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VACCINE THERAPY  
ITS THEORY AND PRACTICE



# VACCINE THERAPY

## ITS THEORY AND PRACTICE

BY

R. W. ALLEN, M.D., B.S. (LOND.)

LATE CLINICAL PATHOLOGIST TO THE MOUNT VERNON HOSPITAL FOR DISEASES  
OF THE CHEST; LATE PATHOLOGIST TO THE ROYAL EYE HOSPITAL;  
LATE GULL STUDENT OF PATHOLOGY, GUV'S HOSPITAL

THIRD EDITION

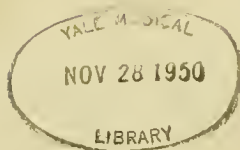
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## PREFACE TO THE THIRD EDITION

THE author trusts that the present edition may be as favourably received as its predecessors ; it has been completely rewritten and brought up to date. The attempt has been made so to render available the experience of the past five years by the inclusion of illustrative cases as to enable the general practitioner to approach with confidence a case requiring therapeutical immunization. Accuracy in diagnosis, close clinical observation, and an open mind, are the prime essentials for success.

128, HARLEY STREET, W.,  
*June, 1910.*



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# VACCINE THERAPY

## CHAPTER I

### OPSONINS: WHAT THEY ARE, THEIR NATURE AND SOURCE

OF the means whereby the body tissues are enabled to overcome bacterial invasion our knowledge is as yet far from perfect. The process is admittedly a very complex one. Various substances, to which the names 'agglutinins,' 'precipitins,' 'stimulins,' 'lysins,' and 'opsonins,' are given, are considered each to play a part in enabling the phagocytic cells to complete the destruction of the infecting bacteria. Metchnikoff holds that the principal part is played by the substances to which he has given the name 'stimulins.' The presence of these in the tissue fluids he has not yet succeeded in satisfactorily demonstrating, but considers their function to be that of acting upon the phagocytes so as to stimulate them to perform phagocytosis. While not denying the existence of opsonins, he assigns to them but a secondary part. Wright, on the other hand, has demonstrated beyond doubt the presence in the blood of substances which act upon the bacteria, and get them ready for the completion of their destruction by the phagocytes. To these bodies he has given the name of 'opsonins.' It would appear possible for phagocytosis to proceed without prior opsonization of the bacteria, unless it be argued—and this seems very plausible—that the phagocytic cells contain opsonins in their plasma fluid from which it is

hardly possible to free them. Be this as it may, it is beyond question that the presence of opsonin materially assists the processes of phagocytosis.

*The method whereby the presence of opsonin in blood-serum is demonstrated* is as follows : A little freshly-drawn blood is immediately received into eight or ten times its volume of 2 per cent. sodium citrate to prevent coagulation. The blood-cells are thrown down by rapidly centrifuging, and the supernatant liquid pipetted off. The cells are then thoroughly washed with a considerable bulk of a solution of 0·8 per cent. sodium chloride in distilled water, and again thrown down by means of the centrifuge, this process being repeated two or three times, so that finally the cells are washed practically free from all blood-plasma, and are left suspended in a very small volume of the normal saline solution, as uniform a mixture as possible being made. A twelve to eighteen hour old culture on agar of any organism—say *Staphylococcus albus*—is then taken, and a thick emulsion made with a solution of 0·1 per cent. sodium chloride in distilled water. Clumps are thrown down by means of the centrifuge, and the bacterial emulsion divided into two parts, *A* and *B*. *A* is set aside ; to *B* an equal volume of fresh blood-serum is added, and the two thoroughly mixed together and heated in an incubator at 37° C. for fifteen minutes. The bacteria are thrown down by means of the centrifuge, and as much liquid as possible pipetted off. The precipitated bacteria are well washed with 0·1 per cent. solution of sodium chloride in distilled water, and again thrown down, this process being repeated several times. Finally, an emulsion of the bacteria is made in the salt solution exactly like *A*, and the numbers present respectively in emulsions *A* and *B* counted, the thicker emulsion being diluted to exactly the same strength as the weaker. We thus

have a suspension of blood-cells of which unit volumes contain the same number of polymorphonuclear white cells—*i.e.*, of phagocytes—and two emulsions of the same strength of a given organism in 0·1 per cent. salt solution, differing only in the fact that the bacteria in one (*B*) have been acted upon by blood-serum at 37° C. for fifteen minutes. If this has had no action upon the organisms, identical results should be obtained by the following procedure: Equal volumes of the blood-cells and the bacterial emulsion *A* are thoroughly mixed together in a capillary pipette and incubated at 37° C. for fifteen minutes, the same being done with the substitution of emulsion *B* for *A*. Films are spread, stained by Leishman's method, and observed under a  $\frac{1}{12}$ -inch oil-immersion lens. The number of bacteria engorged by 100 polymorphonuclear leucocytes is then counted upon each film. An experiment performed in this way gave the following result:

Bacterial Emulsion employed.	Number of Cocci in 100 Polymor- phonuclear Leucocytes.
<i>A</i>	10
<i>B</i>	500

It is thus obvious that some change is produced in the bacteria by the action of the blood-serum whereby phagocytosis is expedited. To the substance by which this change is brought about Wright gave the name 'opsonin.'

#### THE NATURE AND CONSTITUTION OF OPSONINS.

Until they have been isolated and obtained in a state of purity, it is obvious that the exact constitution of opsonins cannot be determined.

Certain observations render the view probable that they are of a proteid nature. Thus, Yorke<sup>1</sup> filtered normal

<sup>1</sup> *Biochemical Journal*, vol. ii., June, 1907, p. 357.

serum through a sterile Chamberland candle under very high pressure, and found that the opsonin passed readily through for the first few minutes, but that after that only traces permeated the candle-wall, owing to the pores being filled up by the proteids of the serum. The residue in the filter, beside containing comparatively unaltered serum, consisted also of a gelatinous substance, adherent to the sides of the candle and of high opsonic power. It would thus appear that opsonins will not pass through a Chamberland candle the pores of which have been blocked up with gelatine or proteid substance. They would therefore appear to be of a 'colloidal' nature. Lamar and Bispham<sup>1</sup> showed also that they were not dialysable, and that they are carried down with the euglobin when serum is half saturated with ammonium sulphate.

In certain respects they bear some resemblance to the ferments. Thus, serum can be diluted to a considerable extent without marked lessening of its opsonic power. Noguchi<sup>2</sup> has also shown that they are not destroyed by drying the serum at 23° C., and that in this desiccated state they retain their activity after two years, and are, moreover, comparatively resistant to heat. Exposure to a temperature of 120° C. but slightly impairs their power, which is not altogether destroyed by a temperature of 150° C.

Like ferments, opsonins are also very sensitive to slight alterations in the acidity or alkalinity of the medium in which they are dissolved, displaying the greatest activity in a solution of neutral reaction. As regards their biological constitution diverse views are held. It would, however, appear that the opsonins present in normal serum and in that of an infected or immunized animal, which is known as an 'immune' serum, are not quite the

<sup>1</sup> *Journal of Experimental Medicine*, December, 1906.

<sup>2</sup> *Ibid.*, vol. ix., No. 4, p. 455.

same thing, and the elucidation of their nature has been much hindered by the failure, especially of the earlier investigators, to recognize this possibility.

The following experiment of Yorke and Smith<sup>1</sup> upon normal serum corrected earlier observations by Bulloch and Western, and has been amply confirmed by other investigators. A strong emulsion was made of anthrax bacilli in 0·9 per cent. NaCl solution, and killed by heating at 100° C. for thirty minutes. The bacilli were thrown down by centrifuge, washed thrice with 0·9 per cent. NaCl solution, the washed dead bacteria made up into a strong emulsion, and added to two equal portions of 'normal' serum, *A* and *B*. *A* was incubated at 37° C. for thirty minutes, *B* for sixty minutes.

The bacteria were then thrown down, and the supernatant sera tested for opsonin with staphylococcus and anthrax. The control sera were diluted to an equal extent with 0·9 per cent. NaCl solution and incubated for similar times with the like organismal emulsions. The figures obtained from the films prepared therefrom were as follows :

TABLE I.

Number of Bacteria phagocytosed by 50 Leucocytes.						Index.
<i>A.—I. ANTHRAX :</i>						
Control serum	..	40	..	..	..	1·00
Treated serum	..	8	..	..	..	0·20
<i>II. STAPHYLOCOCCUS :</i>						
Control serum	..	341	..	..	..	1·00
Treated serum	..	87	..	..	..	0·25
<i>B.—I. ANTHRAX :</i>						
Control serum	..	48	..	..	..	1·00
Treated serum	..	2	..	..	..	0·04
<i>II. STAPHYLOCOCCUS :</i>						
Control serum	..	395	..	..	..	1·00
Treated serum	..	70	..	..	..	0·17

<sup>1</sup> *Biochemical Journal*, vol. ii., December 19, 1906.



Other experiments gave similar results, which have been confirmed by Simon, Potter, and others. It therefore appears that the incubation of a large number of any organism with serum will not only greatly reduce the contained opsonin for that given bacterium, but those for other organisms as well. In other words, much the greater proportion of the opsonin present in 'normal' serum is 'non-specific.' As to how much of the remaining proportion of the opsonin is 'specific,' exact observations are lacking.

Numerous experiments have been performed which demonstrate a difference in the behaviour of the opsonins of 'normal' and 'immune' sera. Thus, Bulloch and Western repeatedly tested the serum of human beings against both staphylococcus and the tubercle bacillus. Injections of tuberculin were then given, and found to produce a rise in the tuberculo-opsonin, while not affecting the staphylococcic opsonin. Injections of killed staphylococci had the reverse effect.

The fact, too, that infected patients are found to have either a high or a low index towards that particular organism, and a normal index towards all others, points to an alteration in the opsonin produced by the infection. That this has resulted in the production of a 'specific' opsonin is rendered probable by numerous observations, of which the following may be given as an example :

If two sera, *A* and *B*, be taken, *A* being a 'normal' serum, *B* that of a person infected by the tubercle bacillus—i.e., an 'immune serum'—and each of these sera be divided into two portions, the one of which is heated at 60° C. for half an hour, the other not, and the opsonizing power of these four specimens of serum towards the tubercle bacillus be estimated, a result like that set out in the following table will be obtained :



	NORMAL SERUM.		IMMUNE SERUM.	
	Unheated Portion.	Heated Portion.	Unheated Portion.	Heated Portion.
Bacteria in 100 leucocytes .. .. .	300	15	200	80

Whereby it is seen that the effect of heating the serum has been very different in the cases of the 'normal' and 'immune' sera respectively. In other words, the amount of 'thermostable' opsonin for the tubercle bacillus is much greater in the 'immune' than in the 'normal' serum.

A comparison of the opsonizing powers towards staphylococcus in the case of these sera would reveal no difference in behaviour.

It is therefore probable that, as a result of the tubercular infection, the amount of 'specific' thermostable opsonin has been increased.

To sum up, while it must be admitted that the demonstration of 'specificity' of opsonins even in 'immune' sera is not complete, it is yet highly probable that in the blood-serum of a perfectly healthy individual there is a minimal amount of opsonin specific against the various pathogenic bacteria, while much the greater proportion is non-specific. The suggestion has been made that opsonin does not so exist in blood or tissue plasma as such, but as 'opsinogen,' needing contact with bacteria or other substances for the formation of 'opsonin,' just as 'fibrin' exists in the blood as fibrinogen, needing calcium salts for its conversion into fibrin. In an individual infected by a given bacterium the amount of opsonin specific against that bacterium undergoes considerable variation from the normal, and is probably increased in every case, not necessarily beyond the amount of 'specific'

opsonin which the healthy individual is capable of elaborating, but beyond the amount which he actually does normally elaborate. Assuming that among the other protective mechanisms of the body a quantity 'A' of 'specific' opsonin is necessary to enable a given individual to overcome an infection by a certain bacterium, his capacity for elaborating this specific opsonin 'A' may be in excess, exactly adequate, or in default. In the first and second instance the infection will be overcome in a time varying, *inter alia*, with the amount of specific opsonin elaborated. In the last instance it will not be overcome until such time as his capacity is raised to the necessary point, or unless the other defensive mechanisms of the body suffice.

As regards the structure of opsonins, the following possible views all have their advocates—

1. That opsonins are identical with certain other immune bodies.

(1) Amboceptors (Savtchenko).

(2) Complements (Levaditi, Inmann).

2. That opsonins are not identical with these other bodies, but—

(1) Have a simple structure like toxins, agglutinins, precipitins, amboceptors, complements ; or—

(2) Have a double structure, like cytotoxins and hæmolysins, needing the co-operation of a thermostable, amboceptor-like body, and a thermolabile, complement-like body (Muir and Martin, Dean, Cowie, and Chapin, etc.).

3. That opsonins are unlike any other antibody, and form a class by themselves (as originally upheld by Wright and Douglas, Bulloch and Atkin, Keith, Hektoen, Neufeld, and others).

To enter into a full discussion of all those possibilities is quite outside the scope of this book ; the theory ren-

dered most probable by the weight of present evidence is the second of these, which may be enunciated as follows : Opsonic action is the effect of two bodies acting together—one, thermostable and of amboceptor-like nature, is the essential substance ; alone, it is perhaps capable of opsonizing, but its activity is greatly increased by the presence of a thermolabile, complement-like body. The amboceptor-like constituent is present only in very small quantity in normal serum ; hence the apparent thermolability of the opsonin in normal serum, whereas in an 'immune' serum the amboceptor plays the predominant part ; and though heating results in a loss of activity, this is only partial. In the case of both normal and immune serum this loss is due to the destruction of the complement-like constituent. Considerable support is lent to this view by recent experiments, and notably those of Cowie and Chapin,<sup>1</sup> whereby they showed—

1. That heated normal serum may be reactivated by the addition of small amounts of fresh normal serum, a phagocytosis resulting which is greater than the sum of the phagocytoses of the sera taken separately. A similar result is obtained with heated 'immune' serum—*i.e.*, the addition of complement in the fresh serum assists the amboceptors of the heated serum.

2. That just as ordinary amboceptors can effect combinations at the freezing-point, while complements cannot, so with opsonins. Thus, normal serum may have its opsonic power for staphylococcus removed by addition of sufficient staphylococci, the mixture being maintained at a temperature near 0° C. throughout.

That this has resulted in the binding of the amboceptor-like constituent while the complement remains free is

<sup>1</sup> *Journal of Medical Research*, October, 1907, and February, 1908, p. 57 and p. 95.

shown by the fact that serum so treated—the bacteria with bound amboceptor having been removed by centrifuge—may still have the power to reactivate a heated serum.

3. Bacteria so treated—*i.e.*, bound to amboceptor—if thoroughly washed with cold salt solution to remove adherent complement, are not much more susceptible to phagocytosis by blood-cells, washed free from complement, than they were before ; but the addition to the mixture of complement, either in a little dilute normal serum or in serum inactivated by contact with bacteria in the cold, results in a phagocytosis greatly above the normal.

4. Staphylococci so treated—*i.e.*, bound with amboceptor—are much more easily opsonized by dilute normal serum, or by serum which has been inactivated by contact with staphylococci in the cold, than are the same bacteria not so treated.

5. That a heated serum loses its power to be reactivated if previously treated with a sufficient number of staphylococci—*i.e.*, if it has its amboceptors thus removed.

Further support is lent to this view by the fact, pointed out by Muir and Martin (1906 and 1907), that in the case of the thermolabile constituent of normal serum various substances which absorb complement also absorb opsonin—*viz.*, erythrocytes, bacilli, and serum—when combined with their corresponding antibodies—*viz.*, hæmolytic and bacteriolytic amboceptors and precipitins respectively—whereas these substances have little or no effect upon the thermostable constituent of an immune serum.

The experiments of Meakins,<sup>1</sup> however, tend to show that in certain instances, at all events, opsonin and amboceptor are not identical, for in the production of active immunity the two may not be increased to anything like the same extent.

<sup>1</sup> *Journal of Experimental Medicine*, January, 1909, p. 100.

## ANTI-OPSONINS : SPECIFIC AND NON-SPECIFIC.

Hektoen and Ruediger<sup>1</sup> have shown that many substances, such as calcium and barium chlorides, sodium bicarbonate, lactic acid, and alcohol, have the power of inducing a marked general fall in the opsonic power of the blood-serum. The addition of any alkali has the same effect as has that of any acid after the alkaline reaction of the serum has been reduced past the point of neutrality. These substances would therefore appear to be non-specific anti-opsonins. Upon the other hand, if a healthy man be injected with antitetanic serum, a specific rise in the tetano-opsonic index first occurs. This is followed, however, by a general fall. Thus, the staphylococcal, tuberculo-, and tetano-opsonic indices all fall below normal (Yorke and Smith).<sup>2</sup> A similar, though less marked, general depression is observed after injection of antistreptococcal or antidiphtheritic serum. R. Bradshaw<sup>3</sup> has recorded the following observations upon the effect of injections of antidiphtheritic serum upon the tuberculo-opsonic index.

TABLE II.

No. of Case.	Interval since Anti- diphtheritic Serum given.					Index.
1	..	..	2 days	..	..	1.30
1	..	..	5 "	..	..	0.64
2	..	..	12 "	..	..	1.02
2	..	..	25 "	..	..	0.35
3	..	..	27 "	..	..	0.89
3	..	..	41 "	..	..	0.72
4	..	..	25 "	..	..	0.64
5	..	..	26 "	..	..	0.72
6	..	..	26 "	..	..	0.62
7	..	..	27 "	..	..	0.77
8	..	..	28 "	..	..	0.69
9	..	..	3 months	..	..	0.47

<sup>1</sup> *Journal of American Medical Association*, May, 1906.<sup>2</sup> *Biochemical Journal*, 1906, p. 341.<sup>3</sup> *Lancet*, May 19, 1906, p. 1387.

Banks<sup>1</sup> found a slight but definite rise in the tuberculo-opsonic index within a few days of the injection of anti-diphtheritic serum, which became converted into a slightly lowered index within a few weeks. The changes were, however, so small as to lie within the limits of experimental error, and, granting that they do represent the actual result, the effect of the serum as serum needs to be taken into consideration.

Other observers do not agree with the observations either of Bradshaw or Banks, but find that an initial fall of slight duration is followed by a pronounced subsequent rise. So much is this the case that marked improvement is claimed to have been observed in tuberculous subjects to whom antidiphtheritic serum has been administered.

The experiments of Hektoen and Ruediger (*supra*) confirm the conclusion that the injection of these sera results in the formation of specific anti-opsonins.

#### SITE OF FORMATION OF OPSONINS.

That opsonin is not formed in the blood is practically certain. The amount of opsonin present in the blood bears no definite relation soever to leucocytosis, nor is it affected by disease of the blood-forming organs. Evidence is forthcoming that it is a product of muscular or subcutaneous activity. Allen has shown both in man and animals that if limbs be thoroughly perfused with normal salt solution to remove all blood, and the muscles cooled and minced and their plasma extracted in the usual manner, that the index of this plasma, despite slight dilution with the saline solution used in the perfusion, is markedly higher than that of the blood-serum towards

<sup>1</sup> *Lancet*, June 26, 1909, p. 1825.



various organisms. In the instance of an amputated leg the index of the muscle-plasma compared with that of the patient's serum was 1·4 towards the bacillus of Friedländer, the tubercle bacillus, and *Staphylococcus aureus*. In another case it was found to be 1·3. From which it may be concluded that actual formation of opsonin occurs in the muscle or subcutaneous tissues, and passes thence into the blood. This local formation explains the experience of Wright<sup>1</sup> that a certain case of tubercular ulceration which had previously defied treatment did well when the tuberculin was injected in a concentric manner around the area of ulceration.

To the bone-marrow and spleen the rôle of opsonin formation has also been ascribed upon somewhat slender evidence.

#### FATE OF OPSONINS IN THE ORGANISM.

As regards this question but little is known. It, however, appears that all exudates and secretions contain certain amounts of opsonin. Miller found that it is contained in appreciable amount in the sweat, and to a larger degree in the urine, and that this excreted opsonin is increased during a negative phase consequent upon the injection of a bacterial vaccine. Milk also contains opsonin, perhaps to the extent of a quarter or a fifth of that of the blood, so that the question as to whether the opsonin of the mother's milk can be absorbed through the alimentary tract of the infant attains considerable importance. Wells,<sup>2</sup> from a study of the indices of breast-fed and artificially-fed infants, has concluded that no advantages in this respect are possessed by the former over the latter.

<sup>1</sup> *Lancet*, August 24, 1907, p. 494.

<sup>2</sup> *Practitioner*, May, 1908, p. 635.

## CHAPTER II

### DETERMINATION OF THE OPSONIC CONTENT OF THE BLOOD

#### DEFINITION OF THE OPSONIC INDEX.

*The opsonic index* may be defined as the ratio :

Opsonic content of unit volume of the patient's blood-serum.  
A normal person's

This is determined according to a method first introduced by Leishman for the estimation of the phagocytic power of blood, as modified by Sir Almroth Wright.

The following materials and apparatus are required :

1. A sufficient quantity of the patient's blood-serum and of that of the normal person.
2. Blood-cells which have been thoroughly freed from the plasma in which they normally float.
3. An emulsion of the bacterium towards which the opsonic index of the patient is to be determined.
4. Glass-tubing  $\frac{3}{16}$  inch and  $\frac{5}{16}$  inch in external diameter—the smaller for collection of the blood samples, the larger for the opsonic determinations. The former are to be cut into lengths of about 3 inches, and drawn out into capillary threads at each end, which are then cut off short. The latter are to be drawn out at one end only into fine capillary threads about 6 inches long and as far as possible of uniform bore.
5. Strong rubber teats, file, grease pencil.



6. Centrifuge with hæmatocrite attachment, and glass tubes to fit the same.

7. Watch-glasses and platinum loop.

8. The following solutions in sterile distilled water, carefully freed from dust and hairs, not by filtering, but by centrifugalization :

(a) 1·5 per cent. to 2 per cent. neutral sodium citrate.

(b) 0·8 per cent. sodium chloride.

(c) 0·1 per cent. sodium chloride.

9. Glass slides thoroughly grease-free.

10. Incubator (Hearson's biological), maintained at 37° C.

11. Methylic alcohol for fixing.

12. Appropriate staining solutions—viz., for all organisms except tubercle, Leishman's stain; for tubercle, carbol fuchsin, 10 per cent. sulphuric acid, absolute alcohol, and toluedene blue.

13. Porcelain jar with metal cover for holding slides during fixing and staining.

14. Microscope with  $\frac{1}{12}$ -inch oil-immersion lens and mechanical stage; cedar-wood oil.

The following procedure is then to be adopted :

1. *Collection of Blood for Serum*.—This is done by cleansing the finger-tip or lobe of the ear with warm soap and water or 2 per cent. lysol solution, drying, and rubbing well with a small piece of lint saturated with ether. When the latter has evaporated, a prick is made with a needle. This is best done decisively, for patients prefer one effective puncture to several ineffective ones. As a rule they prefer the finger-tip to be utilized, but should the epidermis be obviously thick at the root of

the nail, it is better to employ the lobe of the ear. The blood must flow spontaneously, or but very slight pressure be employed, and the first drop wiped away, for, as has been shown, the opsonic content of the plasma of muscle and the subcutaneous tissues is considerably higher than that of the blood. On approximating one of the capillary ends of the tube to the blood, the latter will flow spontaneously into it. Three or four drops of blood will suffice. The tube must now be sealed off, and here a word of caution is necessary; opsonins are readily destroyed by heating to 60° C.; the blood must, therefore, not be heated. All risk of this is avoided by gently warming the end of the tube away from the blood, and then sealing off this end. Lay the tube down flat, and allow it to cool. In doing so the blood is sucked back from the unsealed capillary end by the vacuum produced by the contraction of the contained air as it cools. When this has occurred, that end also may be sealed off in the tip of the flame. These precautions are far from unnecessary, for I have seen many samples of blood quite spoilt in the collecting. The control blood should be taken at the same time as that of the patient.

2. *Preparation of the Blood-Cells.*—The blood for this purpose should not be collected from a sufferer from disease of the lymphatic system, or from an individual whose red blood-cells are capable of agglutination either by their own serum or by that from any other source. As Fleming<sup>1</sup> has pointed out, this is particularly liable to occur in the case of infected individuals, and the effect of agglutination of the red cells in an opsonic mixture is to give an unduly high phagocytic count.

Aseptic precautions should be taken to prevent con-

<sup>1</sup> *Practitioner*, May, 1908, p. 607.

tamination with organisms which will grow rapidly in such a favourable medium, and prove troublesome, perhaps, when the time comes for counting the slides. The collection is done in one of the  $\frac{5}{16}$ -inch glass pipettes, to which a strong rubber teat has been fixed. A little of the sodium citrate solution is first sucked up to prevent coagulation, then the blood, which is at once transferred to a tube containing more of the sodium citrate solution. Blood may be added to the citrate in the proportion of 1 to 5.

The citrate, by precipitating the calcium salts of the blood, effectually prevents coagulation. The citrated blood is now transferred to the centrifuge tubes and thoroughly centrifugalized. A very considerable speed—10,000 revolutions per minute—may be advantageously employed; the corpuscles will be thrown down quickly and yet escape damage. It is to be remembered that the white cells are lighter than the red, and will therefore be thrown down last. It is well to continue the operation till a distinct white layer is seen lying upon the layer of reds, for efficient centrifugalization means numerous white cells, and so greater facilities in counting. The clear supernatant citrate solution is pipetted off, care being taken not to disturb the white layer. Some of the 0·8 per cent. sodium chloride solution is now added to the cells, and these thoroughly mixed up with it and again thrown down. Concentration of the white cells may be effected by removing the upper layer of cells from one tube, adding these to the second tube, the lower layer in the first being then thrown away. The washing with normal saline solution is repeated once or twice. As much of the liquid as possible is finally removed; the cells, thoroughly mingled with what is left, are then ready

for use. A little plug of cotton-wool will prevent access of organisms from the air.

3. *Preparation of the Bacterial Emulsion.*—For this purpose, whenever possible, a culture of the patient's own organism should be employed, for, as Lohlein<sup>1</sup> has pointed out, the individual qualities of a given strain are of marked influence on the resulting phagocytosis. As a rule, the more virulent the bacterium, the greater is its resistance to phagocytosis. Young organisms stain better and more uniformly than old. It is, therefore, better to employ as recent a culture as possible, especially in the case of such organisms as that of Morax-Axenfeld, which begin to involute even before eighteen hours. A twelve- to sixteen-hour-old culture on an appropriate medium—such as agar for staphylococci, streptococci, coli, etc. ; blood-agar for gonococci ; nutrose ascitic agar for *Bacillus Morax-Axenfeld* or *Micrococcus catarrhalis*—is, therefore, to be employed. If fresh blood-serum or ascitic fluid enter, however, into the composition of the medium, it should be heated at 60° C. for half an hour before addition to the agar ; otherwise the organisms cultured thereupon will be subjected to opsonization during growth. If the growth be a very copious one, it is best to take a loopful of the culture on a platinum wire, and carefully emulsify it in a watch-glass with a little of the 0.1 per cent. NaCl solution. If the growth be scanty, then it is best to pour a few drops of the solution into the culture-tube and emulsify it *in situ*. The turbid emulsion thus produced contains many clumps, which are to be thrown down by means of the centrifuge. A minute or two will usually suffice at a high speed, but experience alone will teach just how long it should be

<sup>1</sup> *Ann. de l'Institut. Pasteur*, 1906.

continued. In any case, it must be efficient, for nothing is more annoying than to find clumps in the films when everything has been completed, for if accuracy be desired the whole process must then be repeated. Experience, again, alone will teach whether the emulsion requires further dilution. The opacity of an emulsion, say, of gonococcus must be much greater than that of emulsions of staphylococci or Friedländer's bacillus in order to give the same count in the normals. A strength which will give a count of about 250 to 350 bacteria in 100 white cells of the normal should be aimed at. In the instance of the tubercle bacillus an emulsion once made and found satisfactory may be preserved sealed up in capillary tubes for practically any length of time, especially if the bacteria have been killed by heating to 70° C. for one hour. When wanted, all that is necessary is thoroughly to shake up the emulsion and give it a few sharp turns in the centrifuge to throw down any clumps which may be present.

These preliminaries over, we now take as many of the fine long-drawn capillary pipettes as there are sera to be investigated. They should be chosen of as equal bore as possible. It is advisable for them to have been sealed off at the fine extremity, plugged with cotton-wool at the other, and dry sterilized. The fine ends are cut off square by means of a file scratch, and marks made with a grease pencil about 1 centimetre from the ends. The content as far as this mark is the unit volume in each case. To the plugged ends are fitted the strong rubber teats, and each pipette is marked with a number corresponding to a serum. The rubber teat is now held between thumb and forefinger and gently compressed, the capillary end inserted into the well-mixed blood-cells, and the unit

volume drawn up by slightly relaxing the pressure on the teat. Next a tiny bubble of air is allowed to enter, a second and third volume of blood-cells being drawn up in similar fashion, each separated from the next by a bubble of air. A volume of the bacillary emulsion is now drawn in with especial accuracy, then a bubble of air; finally, 2 volumes of the serum, which must be taken up free from admixture with red cells, as these tend to produce an unduly low phagocytosis. We thus have in order in the pipette 3 volumes of blood-cells, 1 volume of emulsion, 2 volumes of serum, each volume being separated from the adjoining by means of a bubble of air. This is the procedure usually followed, but if the emulsion be suspected to be too thin, then 2 volumes of blood-cells, 1 volume of emulsion, and 1 of serum may be employed, or the original 1, 1, 1 of Wright. The order—cells, emulsion, serum—should, however, always be followed, for in this way contamination of the cells by the bacterial emulsion, or introduction of opsonin from the serum into the emulsion, is avoided. By gentle pressure on the teat the several volumes are expressed on to a clean glass slide, and thoroughly mixed by alternately sucking the mixture into the pipette and squeezing it out again upon the glass slide. Only by thorough mixing can a satisfactory count be ultimately obtained. The mixture is finally withdrawn as completely as possible some little distance into the pipette, and the extremity sealed off in the flame.

This operation is repeated with each serum. The several pipettes, carefully labelled, are placed in the incubator at 37° C. for fifteen minutes. By means of a file-scratch the ends are then cut off, the content of each blown out on to a clean glass slide, and very carefully



mixed. Half the drop is transferred to a second slide, and two blood-films prepared by the slide method—*i.e.*, by drawing the extremity of one slide held at an acute angle over the surface of the other upon which the drop of blood has been placed.

Mention may here be made of two points of some importance: Firstly, the thickness of the blood-film depends partly upon the pressure employed in the spreading, and to a greater extent upon the inclination of the moving slide to the stationary one. The more vertical the former is held the thinner the film, and, conversely, the more acute the angle the thicker the film. Now, the ideal film is one in which the corpuscles do not lie one upon the other, but are even separated by distinct intervals, for in such an one the white blood-cells flatten out, and consequently are of larger size. The contained bacteria are, therefore, much more easily distinguished after staining, and counting is consequently facilitated. Films containing tubercle bacilli may, however, be spread rather thicker than in the case of other organisms, for the staining methods are more drastic, the organisms show up more clearly, and the red cells are practically invisible.

To obtain the best films firm pressure should, therefore, be employed, and the slides should be held at an angle of 60 degrees to one another.

*Secondly*, owing to their greater viscosity, the white cells tend not only to be drawn towards the end of the film, but also to run to the edges. These facts may be turned to practical advantage if the precaution be taken not to place too large a quantity of blood upon the slide. Instead of using a slide of ordinary breadth for spreading, one may be bisected longitudinally by means of a glazier's

diamond, and this half-slide employed. If the drop of blood be then placed at the mid-point of the breadth of the slide, but near one extremity, and the half-slide used as a spreader, a film is obtained with two edges lying some little distance from the margins of the slide, and along these edges the white cells will be found collected. Next, by moving the spreader in a series of little jerks instead of with a uniform motion, a number of little valleys, as it were, are made in the film, in which the white cells collect just as they do along the edges.

Attention given to these trifling details is well repaid by the additional ease with which the slides are counted.

The spread films are allowed to dry in the air; one of each is reserved in case of accident; the others are treated as follows: If containing tubercle bacilli, they are fixed for fifteen minutes in methylic alcohol, or for one hour in a mixture of equal volumes of ethyl alcohol and ether, stained by the Ziehl-Nielsen method, and counterstained with toluidene blue. Five minutes' application of the latter stain, followed by thorough washing under the tap, will show up the bodies of the white cells most effectually. For any other organism than the tubercle bacillus the films are best stained according to Leishman's method.

Next, with  $\frac{1}{12}$ -inch oil-immersion lens and a mechanical stage the numbers of bacteria contained in each consecutive five polymorphonuclear leucocytes are noted till 100 cells have been counted. No estimation can be considered satisfactory unless the numbers of bacteria found in each five cells approximate to each other. The following points may here be noted, and too much stress cannot possibly be laid upon their importance if accuracy be



desired in the estimation: Firstly, the advisability of counting as many cells and their bacterial contents as possible. Reliance is commonly placed upon a count of fifty cells. I would maintain that no amount of care at every stage will insure an accurate result with such a count; 100 cells is the minimum number that should be observed. Secondly, the occurrence of bacterial clumps of any size in a film, especially if these lie upon any of the cells, should damn such a film beyond redemption. There is nothing for it but to repeat that experiment with that serum, and, of course, with a fresh normal. Thirdly, the occurrence of clumps of leucocytes, especially if these be held together by threads of fibrin, should render the experiment null and void. Once more repetition is more than advisable. Of course, both these last difficulties should not occur; they are, as a rule, the result simply of lack of care in preparing the blood-cells and the bacterial emulsion.

The determination of the index is now completed as follows: The normal serum is taken as having an opsonic index of unity. The number of bacteria found in 100 cells of each of the patient's slides divided by the number in 100 cells of the normal slide gives their respective indices. To recapitulate, then, the points of importance, by observance of which accuracy can alone be secured and much time and trouble saved:

1. The solutions used for the preparation of the blood-cells must be quite free from hairs and filaments, for these inevitably entangle the white cells and lead to clumps in the films.
2. The blood must be received into sufficient citrate solution to insure complete prevention of clotting, and the cells, when washing is complete, must be thoroughly

mixed to insure equal numbers of leucocytes in equal volumes.

3. The bacterial emulsions must be thoroughly centrifugalized to free them from all clumps, and growths of not more than eighteen hours should be employed for their preparation. The strength should be such that 250 to 350 bacteria are found in 100 cells of the normal.

4. The several volumes must be thoroughly mixed, both before and after incubation, to secure uniformity of count in each series of five cells.

5. The films must be spread thinly to insure the polymorphs being as large as possible.

6. Staining must be satisfactory, and the cell-body shown up. If this prove not so, the reserve slide must be stained.

7. At least 100 cells in each film should be counted, and to obtain this number it should be necessary to search a considerable area, including both ends as well as the centre of the film.

8. If at the first attempt an unsatisfactory result is obtained, whether from clumps of cells or bacteria, or from too few white cells being present in the films, perseverance in counting the imperfect films is to be deprecated. Time and temper will alike be saved by repeating the whole estimation.

Brief reference may here be made to recent attempts at shortening the technique in determinations of tuberculo-opsonic indices by the employment of emulsions of killed organisms which have been already stained with carbol fuchsin. Although one or two observers<sup>1</sup> have reported favourable results, the more general experience

<sup>1</sup> Campbell, *British Medical Journal*, April 13, 1907, p. 866.

is that accuracy cannot be thereby secured. The chief difficulty appears to be in the preparation of a satisfactory emulsion free from clumps. Staining, whether by weak and cold or hot and strong fuchsin solutions, seems to affect the organisms in such a way that centrifugalization, which throws down the clumps, also suffices to throw down the single bacilli.

#### THE QUESTION OF THE ACCURACY OF THIS METHOD OF ESTIMATING THE OPSONIC CONTENT OF THE BLOOD.

It must be admitted that the reliability of estimations of the opsonic content of the blood, conducted according to the above technique, or slight modifications of it, has been rudely assailed during the past two years—among others by Simon, Lamar, and Bispham,<sup>1</sup> and by Walker<sup>2</sup> in America, and by FitzGerald, Whiteman, and Strange-ways<sup>3</sup> in England.

In these un-Socratic days the honesty of all, both of those upholding and of those opposing any given procedure, is to be assumed. Against their experiments and results, then, are to be placed those of Wright and his co-workers, Bulloch, White,<sup>4</sup> the author, and many others. Upon the one hand are those who obtain unreliable results; upon the other those who truly believe, and, as far as figures can substantiate a belief, find support in their figures for the belief, that in their hands the method affords reliable results.

*Journal of Experimental Medicine*, August, 1906, p. 651; *ibid.*, September, 1907, p. 485.

<sup>2</sup> *Journal of Medical Research*, July, 1907, p. 521.

<sup>3</sup> *Bulletin of Committee for Study of Special Diseases*, Cambridge vol. i., No. 8.

<sup>4</sup> *Practitioner*, May, 1908, p. 639.

It thus follows that there are two classes of observers : (1) those who can estimate an index accurately by these methods ; and (2) those who cannot estimate an index accurately by these methods—just as there are surgeons who can perform perfectly the delicate operations advocated by Mayo Robson and by Arbuthnot Lane, and others who cannot. Upon the one hand, the possibility of the proper performance of these operations cannot be impugned by any multiplicity of ill-results in unskilled hands ; upon the other hand, the possibility of the accurate estimation by this method of the opsonic content of the blood is not disproved in the slightest by any number of inaccurate estimations in unskilled hands.

Let me not be misunderstood. I make no claim that the accuracy of the method is comparable with that, say, of the determinations of the various physical coefficients. There are pitfalls innumerable for the unwary, and even the most skilled experimenter will every now and again obtain a wrong result ; but here is an important point : he will know that he is obtaining an unreliable result, and will either repeat the whole estimation or count a much larger number of cells than usual, and so minimize the error. Fleming<sup>1</sup> appositely remarks : ‘ In this connection it should be pointed out that it is a great mistake to have any arbitrary number of leucocytes which one counts, neither counting more nor less, whatever the conditions may be ’—a remark with which I most completely agree ; and again : ‘ Intelligence must be brought to bear on the subject in hand.’

Despite this, it cannot but be admitted that even in the hands of the most skilled there is, as Greenwood<sup>2</sup> points out, an error inherent in any such method which

<sup>1</sup> *Practitioner*, May, 1908, p. 627.

<sup>2</sup> *Ibid.*, p. 641.

is quite inevitable—an error, however, which only rarely exceeds 10 per cent., and is usually about 5 to 6 per cent., and is of little or no practical importance.

The value of the index thus determined as a guide in immunization is discussed subsequently (see p. 80).

#### OTHER METHODS OF ESTIMATING THE OPSONIC CONTENT OF THE BLOOD.

Inasmuch, then, as the above method of determining the opsonic index must be admitted to be a very delicate and somewhat laborious and difficult matter, and one, moreover, which is unsuited to a certain proportion of workers, any other method which is less laborious, less difficult, and suitable for all, can only be regarded as in the highest degree desirable.

Several attempts have been made in this direction. Thus, Simon (*v.s.*) would substitute an index obtained by diluting the blood in varying proportion (ten to thirty times), and after incubating with a bacterial emulsion of considerable strength, comparing the percentage of phagocytizing leucocytes in the specimen of blood under investigation with the figure obtained after a similar procedure with a specimen of normal blood. This he calls the 'percentage index,' and finds it sometimes to agree well with the opsonic index, sometimes to differ considerably from it, in which event he prefers to follow the guidance of his percentage index. As to the accuracy of the method I can offer no opinion, but it is admittedly even longer than the method it seeks to displace, and to my mind has this very grave objection: the dilution of the serum. It is perfectly true that if the opsonizing power of the serum of an infected individual be com

pared with that of the serum of a healthy individual, marked differences are revealed according as the undiluted sera or the sera in various degrees of dilution are compared. As dilution proceeds, it will sometimes be found that the opsonizing power of the immune serum rapidly falls off in comparison with that of the normal serum. Thus, an index of 1·4 may be obtained for the undiluted immune serum, and an index of only 0·8 for the same serum in a dilution of 1 in 20. Certain observers—as Simon and Walker (*v.s.*), who estimate the opsonic index by means of diluted sera—consider examination of such to afford the better idea of the immunizing power of the blood. I would maintain that they are conducting an investigation—the results of which are doubtless of value—under conditions which do not in the least obtain in any pathological condition in the human organism. If possible, what one desires to ascertain is the opsonizing power, not even of the blood-serum, but of the blood-plasma, in the condition in which it actually is in the human organism. Moreover, not even in a suppurating focus nor in an exudate of pus do the bacteria ever present any such ratio to the phagocytes as they employ in their phagocytizing mixtures. In order to learn the immunizing response of the body, say, in a case of streptococcal septicæmia, where, perhaps, ten streptococci can be isolated from 5 c.c. of blood, they would present an emulsion containing at least 5,000 million organisms per c.c. to a mixture of blood-cells containing, perhaps, 5,000 to 10,000 phagocytes per c.c. in a serum diluted twenty or thirty times. Can any conditions less like these obtaining in the human organism easily be conceived? At the same time it must, however, be admitted that under certain conditions this ‘dilution



method' gives a more accurate idea of the total amount of protective substances present in the blood-serum.

The method suggested by Stewart,<sup>1</sup> Dodds,<sup>2</sup> and Veitch<sup>3</sup> has, on the other hand, much to recommend it, and most nearly of all approaches the natural conditions. It is conducted as follows : One volume of blood is withdrawn from the patient in a sterilized capillary pipette, and at once thoroughly mixed with an equal volume of 2·0 per cent. solution of sodium citrate in 0·8 per cent. salt solution, and the ends of the pipette sealed. The same is done with the control normal blood. These mixtures are preserved till required, and, according to Stewart, keep unaltered for twelve hours, or, if kept in a refrigerator, for three days.

The phagocytic mixture is made by taking two volumes of the blood citrate mixture and one volume of the bacillary emulsion, and proceeding in the usual way. The hæmophagocytic index thus determined agrees well, as a rule, with the opsonic index, while the method affords a certain saving of time, especially when only two or three bloods have to be examined, and is more accurate, inasmuch as clumps of leucocytes very rarely occur. The employment of the blood-plasma instead of serum, and of the patient's own leucocytes, are two important modifications ; for, as Dreyer and Ainley Walker<sup>4</sup> have shown, the plasma is usually more highly agglutinative than the serum and less rich in complement ; inequality of opsonic content in the two is therefore also likely. As regards the leucocytes, Rosenow,<sup>5</sup>

<sup>1</sup> *Journal of Bacteriology*, 1908.

<sup>2</sup> *British Medical Journal*, October 12, 1907, p. 948.

<sup>3</sup> *Journal of Pathology and Bacteriology*, January, 1908, p. 353.

<sup>4</sup> *British Medical Journal*, January, 1909, p. 151.

<sup>5</sup> *Journal of Infectious Diseases*, 1906, vol. iii., p. 683.

Glynn and Yorke,<sup>1</sup> Bushnell, and others, have shown that these are by no means an indifferent factor, the phagocytic power of some being above normal, and of others below. This is especially true in the case of pneumonia, where Rosenow found that the leucocytes were, as a rule, hyperactively phagocytic. Ledingham and Bulloch observed, on the other hand, a marked diminution of phagocytic power in the leucocytes during the period of leucocytosis produced by injection of sodium cinnamate.

<sup>1</sup> *Lancet*, September 19, 1908.



## CHAPTER III

### PREPARATION OF THE VACCINE

THE general consensus of opinion is that the best possible results are, as a rule, only to be looked for when organisms isolated from the patient's own lesion are employed for the manufacture of the vaccine. Several considerations may, however, militate against the advisability of this procedure. The chief of these are as follows :

1. Where the isolation of the organism is so difficult and tedious that the resultant loss of time would fail to compensate for the advantages obtained. An excellent example of this is afforded in tuberculous affections. Here we are compelled to resort to inoculation experiments, the animals usually selected for the purpose being the rabbit or guinea-pig, and the site of inoculation either the subcutaneous tissue of the groin of the latter or the anterior chamber of the eye of the former. Of these two animals, the guinea-pig is generally held to be the more susceptible to the tubercle bacillus, dying of general tuberculosis from six to ten weeks after inoculation, according to the virulence of the organism and the number introduced. On the other hand, if tuberculous material be introduced into the anterior chamber of the eye of the rabbit, an iritis which is almost pathognomonic results in from two to four weeks. In either case the loss of time is very considerable. Nor is this all. The growth of the tubercle bacillus is again so slow, and the preparation of

tuberculin so difficult an operation, that another two or three months would be consumed in the preparation of the vaccine. This is very greatly to be regretted, for many of the only partial successes or even failures in cases treated by tuberculin are possibly very largely due to the employment of stock tuberculin. This question will be again referred to later.

A second example of this class of case is afforded in some chronic gleets. The gonococcus may be visible in smears of the urethral secretion, yet, despite the utmost care in taking the cultures, it may prove impossible to free the gonococcus from the contaminating organisms.

2. The infection, although localized, may be of so acute and destructive a type that the loss of even one or two days may be of vital importance. An excellent example of this is seen in gonorrhœal conjunctivitis in the adult. Here prompt injection of a stock vaccine is obligatory immediately the patient is diagnosed. I have seen cases, so severe that total destruction of the sight was inevitable in two or three days, thereby completely held in check, and, save for the destruction which had already occurred, cured within a week (*vide* chapter on The Eye).

3. Where the infection is so very chronic that it is reasonable to suppose that the virulence of the infection has been greatly reduced, though even here it is better, wherever possible, to test the virulence by an inoculation experiment upon animals. Good examples of this class of case are afforded by—(a) very chronic cases of osteomyelitis which have been subjected to considerable surgical treatment; (b) chronic gonorrhœal infections, especially old gleets in the male, and tubal cases in the female.

4. A final exception may be made in the instance of such organisms as seem to be definite entities, and not to

compose a family group of such closely related members as the streptococci. So far as we are aware, there is, for example, but one *Bacillus septus* and one *Micrococcus melitensis*; yet even here the better plan is undoubtedly the preparation of a special vaccine, unless other considerations are against the adoption of this plan.

The first thing to do is to endeavour to learn the exact nature of the infection. Should it be presumed to be a systemic one, blood-cultures are necessary and should be performed in duplicate, aerobic and anaerobic incubation being employed. The skin over the antecubital fossa of the arm is thoroughly cleansed, first with antiseptic, then with sterilized water. A tight bandage is then applied well above the elbow. Into a 10 c.c. all-glass syringe, which has been well boiled, about 1 c.c. of sterilized 2 per cent. sodium citrate solution is introduced, to prevent clotting in the needle. The vein is then punctured in a direction against the venous flow, when the blood will at once flow into the syringe, which may be slowly filled. Six tubes, each containing 15 to 20 c.c. of broth, are taken; into two 3 c.c., into a second two 1 c.c., and into the third two 0.5 c.c., of blood are introduced. The tubes are then well shaken, and one set of three incubated aerobically at 37° C., the other set anaerobically. Growth may appear in eighteen hours, but should it not do so, incubation should be maintained for three days before a negative result is assumed. Films are prepared and stained by Gram's method, and subcultures planted upon the medium or media which an examination of the films indicates. Should more than one organism appear to be present, then suitable 'plating' methods must be adopted to secure pure cultures.

Should the infection not be a systemic one, but of such a nature that it is possible to secure pus or secretion of some kind from its site, then careful microscopy of films cannot be too strongly urged prior to any attempt being made at cultivation. The reasons for this are several: Firstly, the presence of organisms, such as *B. influenza* or the gonococcus, which occasionally obstinately refuse to grow even upon the most favourable media, will be revealed; secondly, it will often enable choice to be made of the most suitable media and of the best methods of isolation; thirdly, an idea may be formed of the number of bacteria present, and therefore of the amount of secretion necessary to use in inseminating the culture media; fourthly, from the relative numbers of the bacteria present and the character of the cells some useful deduction may be made as to the relative importance of the organisms present in cases of mixed infection, which cultures might fail to furnish.

For the isolation of the bacteria in pure culture the following method will prove almost universally useful: Blood, best taken aseptically from the human subject, is mixed with three parts of sterilized 2 per cent. sodium citrate solution. One c.c. of this is added to 10 c.c. of liquefied agar at a temperature of 50° to 55° C., and plates prepared therefrom in Petri dishes of about 4 inches diameter. The medium should be of a bright pink colour, capable of revealing clearly the hæmolytic action of the pneumococcus or streptococcus.

According to the indications derived from the previously studied film preparations, a portion of the secretion of greater or less size is then taken up with the platinum loop and smeared gently over the surface of the now solidified blood-agar plate. If the organisms be very

numerous, a series of strokes is best made instead of the more uniform smear. The plate is incubated aerobically at 37° C. for eighteen to twenty-four hours, or longer if necessary. Perfectly discrete colonies should then be visible in some portion or other of the plate, if this has been skilfully planted, and can be picked off for subculture. Of course, if anaerobic organisms are to be sought for, anaerobic incubation of a second plate is necessary.

The adoption of this method should practically completely obviate failure to secure growth of any freely-growing organism. Occasionally subplating of the colonies is necessary to insure purity.

The method is applicable to—and, indeed, usually the only one necessary for—the isolation of any of the catarrhal organisms found in sputum, viz., the *B. influenzae*, pneumococcus, *B. septus*, *M. catarrhalis*, *M. paratetragenus*, bacillus of Friedländer, also to the *B. proteus*, *M. tetragenus*, streptococcus, staphylococcus, gonococcus, *B. coli*, *B. typhosus*, meningococcus, *B. pyocyaneus*. Very occasionally a few of these organisms, such as the *B. influenzae* and gonococcus, fail to grow, but when this is so they will probably fail to grow upon any other culture medium. A few other organisms, again, there are to whose isolation this method is not suited. First among these may be mentioned the tubercle bacillus, inasmuch as the author feels convinced that more and more will it be found advantageous in difficult cases to continue the use of a stock tuberculin only while a special one is in process of manufacture.

The peculiarity of localized tubercular affections is the paucity in the number of bacilli present. This holds true

whether it is the pus from the tubercular joint or the tissues of a tubercular gland or conjunctiva. In the case of tubercular bladders and kidneys, however, very large numbers of bacilli may be discharged in the urine. Some means must therefore be adopted not only of freeing the tubercle bacilli from contaminations, but also for increasing their numbers. This is done by inoculating a rabbit or guinea-pig. If the material be pus or solid gland, this is done directly; if conjunctiva, care must be taken to cleanse the eye thoroughly with sterile saline before removing the piece of conjunctiva; if pus in urine, the bacilli are to be separated by thoroughly centrifuging the urine which has been drawn off with a catheter, washed well with sterilized water, and again centrifuged, repeating this several times. The final deposit may then be employed like any other inoculum. Immediately the animal has died—or as soon as caseous glands are to be felt, or after three to four weeks in the case of injection into the anterior chamber of the eye of the rabbit, death being produced by means of chloroform—it is opened with all antiseptic and aseptic precautions, and culture-tubes of glycerine egg medium or glycerinized potato inseeded with as large portions of the diseased glands or iris as is possible. The tubes are then sealed up and incubated at  $37^{\circ}$  C. for six to eight weeks, when a copious growth of tubercle bacilli should be available for the manufacture of tuberculin. Unfortunately, there are cases where the tubercle bacilli thus obtained, often in considerable numbers, from the glands and spleen of the injected animal, refuse to grow in artificial cultures. The following modification of an old method has been given me by Williamson as very useful in obtaining cultures direct from sputum: A suitable lump



is chosen, washed thoroughly in sterile salt solution, removed therefrom, and mixed with four parts of glycerine, which is heated in a water-bath at 60° C. for five minutes. After incubation at 37° C. for ten to fourteen days the tubercle bacilli are thrown down with the centrifuge, washed with sterile salt solution, and subcultured upon glycerine egg medium or glycerine nutrose agar.

Among some other organisms in the case of which special methods are necessary may be mentioned: (1) The acne bacillus; (2) the bacillus of Morax-Axenfeld; (3) the *Bacillus typhosus* and its allies.

*The Acne Bacillus.*—Recent investigations by Fleming,<sup>1</sup> Gilchrist,<sup>2</sup> and others, have confirmed the observations made by Unna in 1893, and by Sabouraud in 1897, and have established a definite causal relationship between this bacillus and many cases of acne. The acne bacillus is best obtained from the deeper parts of the sebaceous plugs, but also from many pustules, by anaerobic incubation in broth for ten to fourteen days. Most of the accompanying staphylococci have by then died out, while the acne bacilli have multiplied. To 10 c.c. of melted 3 per cent. agar, rendered acid + 30 (Eyre's scale) by means of lactic or hydrochloric acid, 5 c.c. of fresh sterile ox or sheep serum are added, and a plate poured in a Petri dish of 4 inches diameter. A loopful of the broth culture is spread over the surface of the plate, which is incubated, best anaerobically, for three days, when little difficulty should be experienced in picking off the colonies of the small Gram-positive diphtheroid acne bacillus. These may then be inseminated upon fresh plates or

<sup>1</sup> *Lancet*, April 10, 1909, p. 1035.

<sup>2</sup> *Johns Hopkins Hospital Reports*, vol. ix.

sloped tubes of the same medium, and should grow fairly readily if incubated aerobically.

*The Bacillus of Morax-Axenfeld* is best isolated by taking a loopful of the thin secretion from near the inner caruncle of the eye of a case of chronic conjunctivitis, and spreading it over the surface of a plate of solidified sheep or ox blood serum. After twenty-four to thirty-six hours' incubation, characteristic areas of liquefaction of the blood-serum will be evident, and from the bottom of one of these material may be taken to inseminate tubes of nutrose ascitic agar (3 per cent. agar, in which 2 per cent. nutrose has been dissolved, and to which one part of ascitic fluid has been added).

*The Bacillus Typhosus and its Allies.*—When these are present in urine or fæces, considerable difficulty may be presented to their isolation. Jackson and Melia<sup>1</sup> recommend incubation of the material for forty-eight hours in lactose bile medium—*i.e.*, sterilized undiluted fresh ox bile, or 11 per cent. solution of dry fresh ox bile to which 1 per cent. peptone, 1 per cent. agar, and 2 per cent. lactose, have been added. Subcultures are then prepared upon large plates made of Hesse agar (for preparation of which see *loc. cit.*). Upon these the typhoid and colon bacilli can be differentiated, and colonies picked off to plant subcultures.

#### PREPARATION OF THE EMULSION.

Having thus obtained a pure eighteen- to twenty-four-hour-old growth of the organism on the suitable medium, we now proceed as follows (the tubercle bacillus alone excepted). The following are the necessary materials: Beat aluminium or glass rod; 0.1 per cent. solution of

<sup>1</sup> *Journal of Infectious Diseases*, April 1 1909, p. 194.



sodium chloride in distilled water, sterilized by boiling ; two Ehrlenmeyer flasks of 50 to 75 c.c. capacity, and a few small glass beads, also sterilized ; a centrifuge with 15 c.c. centrifuge tubes ; a sterilizer which can be maintained at any temperature between 50° and 60° C. for one to two hours ; tricresol ; a capillary pipette, with rubber teat ; some sterilized solution of 2 per cent. neutral sodium citrate in distilled water ; four glass slides ; Leishman's stain ; distilled water ; microscope with  $\frac{1}{12}$ -inch oil-immersion lens and mechanical stage.

If the culture tube do not contain sufficient water of condensation, 4 or 5 drops of the 0·1 per cent. saline solution are now introduced. By means of the aluminium rod the bacterial growth is emulsified as thoroughly as possible.

The two or three tubes are treated in this way, some more saline added, and the whole transferred to the small flask with a few glass beads. The tubes are washed out with a little saline, which is added to the first portions. The emulsification of the growth may be assisted in cases of great difficulty, as with the *M. catarrhalis*, by heating to 56° C. for two to three minutes, and is completed by agitating the flask for some minutes, the beads helping to break up the colonies present. The emulsion, which should measure about 5 c.c., is now transferred to one of the centrifuge tubes, an equal volume of water being added to the other as counterpoise. After a few minutes' more or less vigorous centrifugalization, according to the size of the organism, the emulsion is pipetted off from the sediment into the second flask, and is ready for standardization.

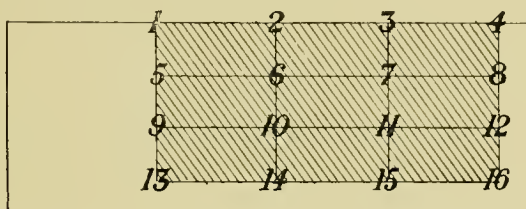
## STANDARDIZATION OF THE VACCINE.

This is carried out as follows : One end of a few inches of glass tubing,  $\frac{3}{8}$  inch in external diameter, is drawn out into a fine capillary thread, which is then cut off, giving a total length of 6 to 8 inches. A rubber teat is fitted to the larger extremity, and a mark made upon the capillary thread about  $\frac{1}{2}$  inch from the end. This constitutes the unit volume. The emulsion being ready to hand, the finger-tip is pricked on the dorsum, and a drop of blood expressed. By gently compressing the rubber teat, and then slightly releasing the pressure, two or three volumes of the 2 per cent. citrate solution are sucked into the capillary thread ; then a small bubble of air, next a volume of blood, then a bubble of air, finally a volume of the emulsion. The whole is then expelled on one of the clean glass slides, and carefully mixed by alternately sucking it up and expelling it upon the slide. This mixing having been thoroughly carried out, the whole is divided into approximately three parts, which are transferred each to a clean slide, and then carefully and evenly spread by means of the edge of another. In this way uniform smears are obtained. These are allowed to dry in the air, and stained with Leishman's stain for five minutes. The slides are then flooded with distilled water, which is allowed to remain for fifteen minutes ; they are then washed in distilled water till pink in colour, and no more blue escapes into the water, and dried with filter-paper.

They are now ready for counting. By means of a blue grease-pencil the two diameters at right angles are marked upon the ocular of the microscope, so that the field is divided into four quadrants. The counting is

thereby greatly facilitated, and is carried out as follows : The smear in the slide is mentally divided up into nine equal areas, as in the subjoined figure. A whole field of the microscope is counted at each of the angles as indicated, so that a total of sixteen fields is counted.

The numbers of red blood-cells seen in each field are set down in one vertical column, the



numbers of organisms in another. Each column is then added up, so that the numbers of corpuscles and bacilli respectively in sixteen microscope fields are estimated. This is repeated for the second slide, and the two results added together. These thirty-two fields may be assumed to give a sufficiently accurate count. We will assume that 600 red cells have been counted and 1,500 bacilli. Now, a cubic millimetre of blood contains 5,500,000 red cells, and equal volumes of blood and of emulsion were taken. A cubic millimetre of the emulsion, therefore, contains  $\frac{5,500,000 \times 1,500}{600}$ , or 13,750,000 organisms per cubic millimetre, or 13,750,000,000 per cubic centimetre.

#### STERILIZATION AND TUBING OF THE VACCINE.

Recent investigations have shown that the method of sterilization of the vaccine is of very considerable importance. Thus, Semple and Matson<sup>1</sup> have shown, in the case of typhoid vaccine, that if heat be employed at all, the

<sup>1</sup> *Lancet*, August 14, 1909, p. 436.

vaccine will not possess immunizing properties of value after six months, whereas a similar vaccine, differing only in the fact that sterilization had been secured by the addition of 0·5 per cent. of pure carbolic acid instead of by heat, remained perfectly efficient three years after preparation. Harrison has also shown, if the vaccine be subjected to a temperature over 53° C., that its efficiency is very much diminished.

My own practical experience tells me that this does not hold equally for all, or indeed for most, vaccines ; but at the same time there is no doubt that the lower the temperature employed for sterilization, the better. It is probable that for most strains even of the streptococcus and staphylococcus the addition of 0·5 per cent. carbolic acid or of 0·3 per cent. tricresol would secure sterility in two to three days ; but careful experimentation upon this point is still lacking, and for the present it is well to adopt the following procedure : Place the flask containing the now standardized bacillary emulsion in a water-bath at 54° C.—seeing that the immersion is complete—for one hour. Remove, and allow to cool. If the strength be sufficiently great to allow of such dilution, add an equal volume of 0·6 per cent. solution of tricresol, and employ a solution of the latter of strength 0·3 per cent. in 0·1 per cent. solution of sodium chloride in distilled water for further dilution as required.

Inasmuch as the amount of local reaction consequent upon the injection of a bacterial vaccine is a factor of the volume injected, it is well to keep the latter down as much as possible. A very convenient amount is 0·7 to 0·8 c.c., and in tubing a vaccine I now always calculate the number of organisms in this volume, and dilute accordingly.

A point worth remembering is that it is almost impossible to foretell how long a case may take to get well. It is therefore wise—(1) to make adequate vaccine to begin with, lest it be impossible to secure fresh cultures later, although recovery be not complete; (2) not to dilute down the whole of the stock, but to put away in a sealed bottle a few c.c. of full strength, lest the supply of large doses prove inadequate. In past years I should at times have been saved great trouble by recognizing these possibilities. Let it be supposed that the highest dose contemplated as necessary is 1,000,000,000, and that some of the vaccine has been reduced to this strength.

We now require a stock of 1 c.c. sterilized glass bulbs of different colours—white, green, blue, brown, amber—or else of one colour, and a diamond pencil wherewith to indicate the dosage upon the bulb. A standard burette graduated in tenths of a c.c. is set upright, and to the lower end a piece of glass tubing, so drawn out as to be capable of entering the necks of the bulbs, attached by means of rubber tubing. The emulsion is then poured into the burette, and the tap of the latter opened till a drop appears at the end of the attached glass tubing; into each of the desired number of white bulbs 0·8 c.c. of the emulsion is placed; the balance is then run off, diluted with an equal volume of 0·3 per cent. tricresol solution, and returned to the burette, the desired number of 500,000,000 doses being filled into green bulbs, and so on. The ends of the bulbs are sealed off in the tip of the flame of a Bunsen burner, and the tubed vaccine set aside, if possible, for two days. If a dose be urgently required, a tube containing the desired strength is again sterilized in the water-bath at 54° C. for thirty to forty

minutes. After the lapse of the two days, one of the highest doses is taken, and its sterility or otherwise ascertained by culture upon a suitable medium. If the contents prove sterile, all is well; if not, then a fresh test may be made after a few more days, or each subsequent dose may be sterilized for thirty minutes in the water-bath at 54° C. just prior to use.

Not only would it appear that the permanence of the immunizing power of the vaccine is increased by avoiding overheating, but the toxicity also seems to be kept within its lowest limits.

#### ADMINISTRATION OF THE VACCINE.

1. *Subcutaneously*. — As there is usually a certain amount of local inflammatory reaction produced at the site of inoculation, it is well to endeavour to secure that the discomfort shall be as slight as possible. To this end it is well to select a site upon which there is little pressure, and where the subcutaneous tissues are abundant, so that the resultant œdema will not cause undue tension. The edges of corsets must especially be avoided, and parts upon which the patient reclines. The most suitable site, especially in males, whose subcutaneous tissues are abundant, is perhaps the flank; next to this, high up on one buttock. Three inches beneath the centre of the clavicle is much recommended by Semple, especially for antityphoid inoculation. In women, also, the flank is to be recommended; but when this is impossible, about  $\frac{1}{2}$  inch from the spine, opposite the angle of the scapula, may be selected.

I am entirely opposed to inoculation being performed into the tissues of the arm or lower leg, and have heard of several cases of extremely severe œdema which have resulted therefrom. The site having been selected, the



skin over it is carefully cleansed with soap and warm water or with 2 per cent. lysol. The neck of the bulb is scratched with a file and broken off, and the inoculum sucked up into a 1 c.c. all-glass hypodermic syringe which has been sterilized, the glass parts by boiling in water, the needle in absolute alcohol. Any air-bubbles are expelled from the syringe, a good fold of skin and subcutaneous tissue taken up between the thumb and forefinger, and the needle plunged deeply into the subcutaneous tissues—not so deep, however, as to cause the inoculum to lie beneath a layer of fascia, or the local pain will be greatly increased. The vaccine is expelled, and the puncture closed with a little collodion.

These aseptic precautions may be considered hardly necessary, but infection from the skin or a dirty needle has been known to occur, and no care is too great to take to obviate such an unhappy result.

*Effects of the Injection—(a) Local.*—It is quite impossible to predict how much local reaction may result. Sometimes there is none at all, especially in well-covered people; at other times there is a little local tenderness, which comes on in six to eight hours, reaches a maximum in twelve to fifteen hours, when the skin around the puncture may be rosy red, and passes off in twenty-four to thirty-six hours, except perhaps for a little dull ache. At other times, but fortunately rarely, an area of skin for several inches around the site of inoculation becomes apparently acutely inflamed and intensely tender—symptoms which may take two or three days to subside.

*(b) Constitutional.* — Usually these are almost negligible, but occasionally are quite noticeable. Within twelve to twenty-four hours there may be a rise of temperature—rarely above 100° F.—some slight headache

and malaise. After twenty-four hours these should pass off, the temperature falling to normal, or, if pyrexia previously existed, showing a marked fall from its prior level. A slight rise of temperature in apyrexial cases—say of  $1^{\circ}$  or  $1.5^{\circ}$  F.—is by no means a bad sign, especially if the infection be a widespread one, as in gonorrhoeal arthritis; in pyrexial cases, on the other hand, it rather indicates overdosage, a fall within twelve to eighteen hours being a much better sign of proper immunizing response.

The question may be asked, Do no untoward results ever occur after an inoculation properly performed? Unfortunately, the answer must be 'Yes, but extremely rarely,' as the following case will show:

A patient suffering from acute suppurative acne was injected in the arm with a staphylococcal vaccine prepared most carefully, and subsequently proved to be sterile. The skin was most carefully cleansed, the syringe well boiled, and the puncture made through a drop of pure lysol. Several days later an abscess appeared, and severe cellulitis of the arm followed. In the pus the *Staphylococcus albus* and what appears to have been the acne bacillus were seen, and the former cultured.

Upon the sterility of the vaccine and the antiseptic and aseptic precautions no doubt can be thrown. The explanation of events would appear to me to be as follows: In the dense tissues of the lower arm a severe œdema resulted from the somewhat high dose of vaccine employed. This latter produced a great lowering of the resisting-power of the adjacent tissues akin to anaphylaxis; these then became auto-infected, possibly through the skin to which the pure lysol had been applied.

2. *By the Mouth.*—Patients, even the best of them, certainly tend to get weary of subcutaneous injections,



especially at diminishing intervals. Latham<sup>1</sup> has accordingly advocated their oral administration. He states that vaccines if given on an empty stomach, with 10 c.c. either of normal saline or of horse serum, are perfectly absorbed, and produce exactly similar results to when introduced subcutaneously. The dosage is approximately double that given by the mouth. Favourable results were reported in staphylococcal, streptococcal, pneumococcal, and tuberculous infections.

Tuberculin may be given in doses of 0·000002 c.c. thrice daily for three or four days, this being the more appropriate where slight degrees of pyrexia are present, or in doses of 0·00002 c.c. every three or four days, which is the better practice in cases where carefully graduated exercise produces an occasional rise of temperature to 99° F., indicating hyper-auto-inoculation. Latham states that when tuberculin by the mouth has a beneficial effect upon the temperature it produces a subnormal temperature, but that not infrequently the chart shows an absolutely normal temperature between 98° and 98·4° F., with disappearance of the previously well-marked amplitude.

This method cannot yet be said to have had its utility satisfactorily established. Possibly it will be found to be of distinct value, but when consideration is made of the cases of antral disease, of chronic catarrhs, and even of pneumonia, which constantly swallow their own organisms, and yet fail to immunize themselves, though they will respond readily to minute doses of vaccine subcutaneously administered, grave doubts must be cast upon the method.

Whether absorption of the vaccine can be improved by

<sup>1</sup> Proceedings of the Royal Society of Medicine, 1908, vol. i., Nos. 6 and 8.

the method shown to be efficient in the oral administration of antidiphtheritic serum by McClintock and King,<sup>1</sup> future experiment must determine. In order to neutralize the acid in the stomach, a glassful of 1 per cent. sodium bicarbonate solution in water is given half an hour before the vaccine, which is combined with 2 to 5 minims of tincture of opium and 10 to 20 minims of a saturated solution of salol in chloroform.

The rectal administration, as advocated by Calmette and Breton,<sup>2</sup> is much too uncertain, and is best left entirely alone.

<sup>1</sup> *Journal of Infectious Diseases*, February 18, 1909, p. 64.

<sup>2</sup> *Comptes Rendus de la Société de Biologie*, vol. lxix., February 1, 1908, p. 163.

## CHAPTER IV

### THE OPSONIC INDEX IN HEALTH AND DISEASE, AND ITS VALUE AS A GUIDE IN VACCINE THERAPEUTICS

#### THE OPSONIC INDEX IN HEALTH.

BULLOCH determined the indices towards the tubercle bacillus for forty-four medical students and forty hospital nurses, all presumably free from tubercular infection. The results showed a variation from a minimum of 0·8 to a maximum of 1·2 as compared with an index of unity for the serum of himself. The average for the whole eighty-four cases was 0·96. Urwick in twenty cases obtained an average of 1·006, and Lawson and Stewart in twenty-five cases an average of 1·0.

The tuberculo-opsonic index of the average healthy individual should therefore lie between 0·8 and 1·2, approximating as closely as possible to 1·0.

The mean staphylococcal opsonic index of twenty-five healthy adults was found by Bulloch to be 1·0 ; other observers have obtained a like result, the variation being, as a rule, less than in the case of the tubercle bacillus. Numerous observations with other organisms show that the same holds true in each case ; it may therefore be assumed that the opsonic index for any organism of the serum of the average healthy person varies only between narrow limits, the minimum being 0·8 and the maximum 1·2.

The index has also been shown by Urwick to be practically constant from day to day in healthy subjects. He gives the following figures for the tuberculo-opsonic indices of the serum of a healthy individual as compared with his own on various dates :

TABLE III.

November 1	=	1.1	December 5	=	0.9
„ 8	=	1.0	„ 8	=	0.9
„ 12	=	1.0	„ 13	=	1.0
„ 30	=	1.15	„ 14	=	1.0
			„ 19	=	1.0

Certain factors do, however, produce very slight changes in the index. French, for instance, has found that vigorous exercise, such as a twelve-mile walk undertaken by a healthy person of sedentary habit, will sometimes cause a rise from 1.0 to 1.2 or 1.3 on the following day. Ellett<sup>1</sup> showed that this positive phase was preceded by a negative one. I myself have noticed a diurnal variation very similar to that exhibited by the temperature chart. This is well seen in the following table, the organism employed being the bacillus of Friedländer :

TABLE IV.

Date.	8 a.m.	9 a.m.	11 a.m.	4 p.m.	12 mid- night.	3 a.m.
May 15, 1906 ..	1	1.06	1.14	—	—	—
May 18, 1906 ..	1	1.11	1.20	—	—	—
May 29, 1906 ..	1	1.08	1.08	1.2	1.26	—
June 6, 1906 ..	1	—	1.07	—	1.18	1.0
June 12, 1906 ..	1	1.10	—	—	1.14	—

<sup>1</sup> *British Medical Journal*, July 21, 1907.

From this it would appear that the index is at a maximum between 4 p.m. and midnight, being raised by the active processes of life, a fall to unity rapidly occurring after retirement to bed. Abstinence from food or excessive exercise did not appear to have any immediate effect in the production of a lowered index.

Charteris<sup>1</sup> made observations upon the opsonic index towards staphylococcus and *B. typhosus* of the blood of a man undergoing a fast of fourteen days, during which no food at all was taken. From counts made upon thirty cells he concluded that the index remained practically unaffected by the fast.

#### THE OPSONIC INDEX IN INFANCY.

Wells and Freeman<sup>2</sup> have made a considerable number of observations upon the opsonic index towards various organisms of the blood of infants from birth up to one year of age. They found practically a normal index at birth, but that it was subject to very great fluctuations from time to time, and concluded—

1. That a low opsonic index is not diagnostic in children under one year old.

2. That a low opsonic index in infants is not inconsistent with health, and that a child may be thriving well with a declining index.

3. That the antibacterial defence in infants cannot depend upon the opsonic content of the serum.

4. That as regards opsonic index the healthy breast-fed infant possesses no apparent advantage over the healthy artificially-fed child.

<sup>1</sup> *Lancet*, September 7, 1907, p. 685.

<sup>2</sup> *Practitioner*, May, 1908, p. 635.

## THE OPSONIC INDEX IN DISEASE.

*The Relationship of Infection to the Opsonic Index.*—Whether fall of index be antecedent to or the result of infection, it is as yet impossible to say, but the following observation clearly shows that infection and lowered opsonic content of the blood may go hand in hand. A case of chronic cold due to Friedländer's bacillus that had been injected some time previously with the corresponding vaccine had an index of 2·6. Twelve hours later an acute attack began to come on with sneezing and shivery feeling. A specimen of blood was taken, and the index found to have fallen to 2·0. Prompt treatment was adopted, and an injection of vaccine given, which stopped further progress of the oncoming cold.

That the fall of index is antecedent to, and not the result of, infection is rendered highly probable by the recent demonstration, referred to on p. 11, of the existence of specific and non-specific anti-opsonins, often of a simple chemical constitution, and by the following consideration: Many people, otherwise perfectly free from acne, frequently develop a crop of pustules when suffering from constipation. Their resistance—*i.e.*, their opsonic index to staphylococcus—is usually normal, but may be assumed to be so lowered by the absorption of toxins—*i.e.*, of anti-opsonins—from the bowel that infection then occurs. Should the index remain low, the acne may persist; should it rise to or over normal, then recovery soon ensues.

*Per contra*, the throwing off of an infection may be accompanied by rise of index. An old sufferer from chronic colds, who had been infected a month previously, had an index to Friedländer's bacillus of 1·5. All the symptoms of a fresh cold appeared, but, as the patient said, he felt he had the cold beaten from the start, and little wonder,

for in twenty-four hours the index rose to 5·8, and the patient was perfectly well.

### 1. IN TUBERCULAR INFECTIONS.

Wright, in his earlier experiments upon localized tubercular infections, found a general lowering of the opsonic index below unity. In a series of seventeen cases, exclusive of pulmonary phthisis, he found variations from 0·4 to 0·85, with an average for the seventeen of 0·64.

Bulloch investigated the indices of 150 sufferers from lupus in all stages, from very mild cases to old intractable ones of even forty years' standing. Seventy-five per cent. of the cases had indices below 0·8, while the average for the 150 cases was 0·75, distributed as follows :

TABLE V.

Opsonic Index.				Number of Cases.	Percentage.
Between 0·2 and 0·3	..	..		3	2·0
„ 0·3 „ 0·4	..	..		3	2·0
„ 0·4 „ 0·5	..	..		21	14·0
„ 0·5 „ 0·6	..	..		29	19·6
„ 0·6 „ 0·7	..	..		33	22·0
„ 0·7 „ 0·8	..	..		22	14·8
„ 0·8 „ 0·9	..	..		18	12·0
„ 0·9 „ 1·0	..	..		7	4·6
„ 1·0 „ 1·4	..	..		14	9·3

In chronic cases of surgical tuberculosis, such as of the joints, kidneys, bladder, or glands, it appears to be generally low, an average of 0·6 being obtained by Bulloch in eleven cases, and of 0·8 in nine cases by French.

Lawson and Stewart made between 2,000 and 3,000 observations upon cases of apyrexial phthisis, and found the index to be always below 1·0, varying from 0·5 to 1·0.

Urwick examined thirty-three cases of pulmonary

tuberculosis in all stages. In twenty-five he found an index above 1.0, even as high as 2.6; in seven an index below 1.0; and in one case the index was 1.0.

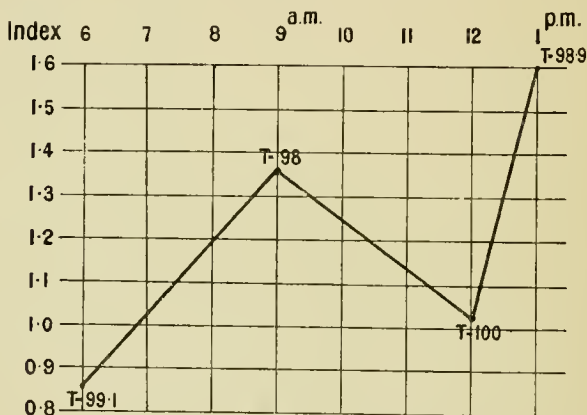
#### EFFECT OF EXERCISE UPON TUBERCULO-OPSONIC INDEX IN CASES OF PHTHISIS.

These variable results were explained by Meakin and Wheeler,<sup>1</sup> who studied the index at various times of the day in tubercular patients who were undergoing sanatorium treatment, some of whom were taking walking exercise and others not; specimens of blood were taken at 6 a.m., 9 a.m., noon, and 1 p.m. If the patient was capable of taking exercise, this was done between 9 a.m. and noon; between noon and 1 p.m. rest was taken in a long chair.

The results of some of their observations—five upon patients taking exercise, and three not—are shown in the adjoined charts:

#### MEAKIN AND WHEELER. WALKING CASES.

CHART I.

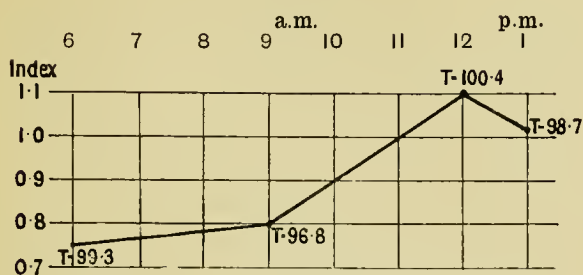


Case No. 1. Walking. September 18.

<sup>1</sup> *British Medical Journal*, November 25, 1905, p. 1396.

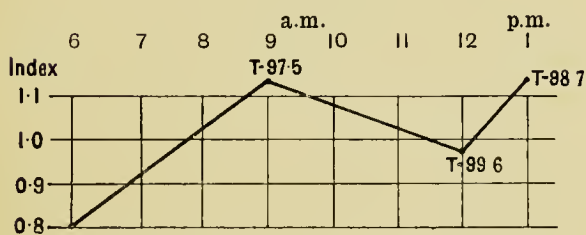


CHART II.



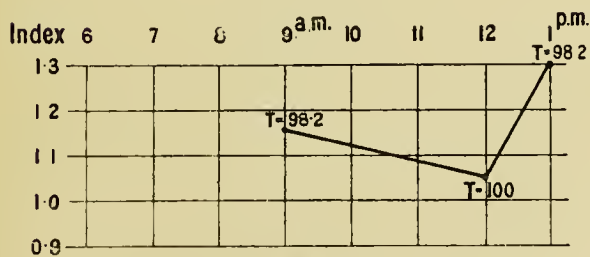
Case No. 2. C. T. Walking. September 27.

CHART III.



Case No. 3. W. L. Walking. September 28, 29.

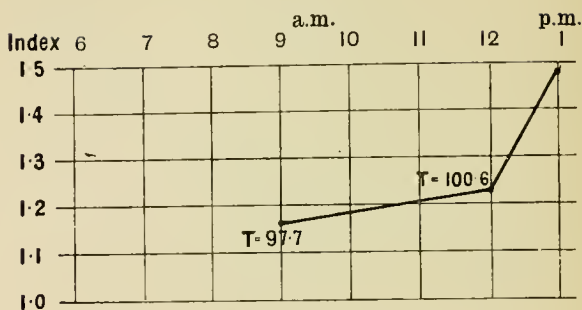
CHART IV



Case No. 4. R. C. Walking. October 11.

## VACCINE THERAPY

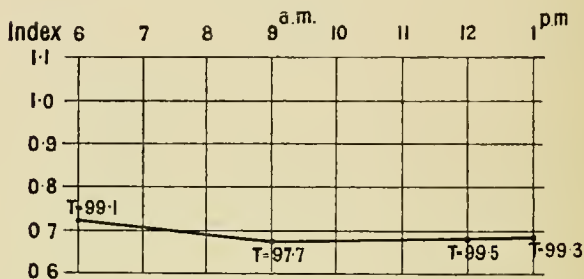
CHART V.



Case No. 5. H.M. Walking. September 16.

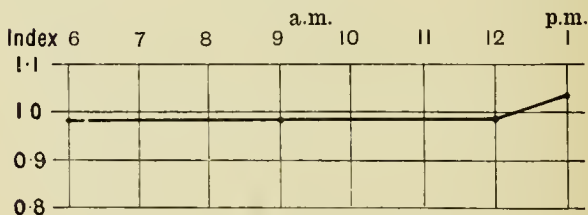
## MEAKIN AND WHEELER. RESTING CASES.

CHART VI.



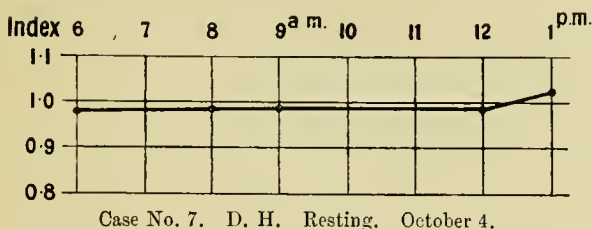
Case No. 5. H. M. Resting. October 1.

CHART VII.



Case No. 6. H. B. Resting. September 18.

CHART VIII.



It will be observed that in all the walking cases except one a much higher index was recorded at 1 p.m. than at 9 a.m., whereas in resting cases the index is practically constant, and either at or below unity throughout the day. This is taken to indicate that in walking cases there occurs a process of auto-inoculation by absorption of extremely minute doses of tubercular toxin by the very vascular lung tissue. How minute the dose is, the short duration of lowered index (two or three hours) clearly indicates. The process thus exactly resembles the succession of negative and positive phases, which, as we shall see (p. 64), is induced by a series of tuberculin injections.

Confirmation of this view is afforded by the like results which we shall see later are produced by surgical manipulation of a tubercular joint. It would thus appear to be generally true that in pulmonary phthisis—

The index is above 1 in slight early cases.

The index is variable in acute cases.

The index is below 1 in chronic cases.

In acute cases there may be either a constant high index, where the body is making every effort to cope with the invaders and the conditions are favourable ; or fluctuating, where auto-inoculation is occurring—not, as

Wright suggests, with too large or badly interspaced doses, but with infinitesimally small ones, so that negative and positive phases are alike of short duration, though full amplitude; or, finally, it may be constantly below unity, if such auto-inoculations be prevented.

In tubercular infections of the eyeball, uncomplicated by tuberculosis elsewhere, the index is usually high. Thus, a case of tubercular iritis had an index of 1·3, a case of keratitis an index of 1·5. A third case, in which tubercular keratitis and iritis were complicated by tubercular cervical and mesenteric glands and by peritonitis, had, on the other hand, an index of 0·4. As will be mentioned later, all eye infections, whether acute or chronic, are usually attended by high opsonic index. The explanation of this is, I think, fairly obvious. The circulation of the eyeball is so poor and the infection so localized that the very minute doses of toxin absorbed act exactly like repeated injections of infinitesimally small doses of tuberculin — so small that the protective mechanism of the body is not exhausted. That chronic cases do not get well with this high index is, again, probably due to the poor circulation, and consequently to the small amount of opsonin brought to the part.

Eyre has observed that broken-down phlyctenules may be starting-points for tuberculosis of the conjunctiva, while Wright, in his earlier experiments, noticed that occasionally phlyctenules developed in patients undergoing inoculations with tuberculin. Nias and Paton<sup>1</sup> accordingly investigated the tuberculo-opsonic index in a series of twenty cases of early phlyctenular conjunctivitis, employing five cases of other forms of conjunctivitis as controls. They found striking variations from

<sup>1</sup> Trans. Ophth. Soc., November 9, 1907.

the normal in the indices of the cases of phlyctenular conjunctivitis, and practically normal indices in the other forms. The author has obtained similar results, but it must not be lost sight of that phlyctenules usually make their appearances in definitely tuberculous subjects, and that the disturbance of the index is probably in the major part due to infected glands, lungs, bones, or joints.

## 2. IN OTHER INFECTIONS.

(a) *In Acute Cases*.—What has been said of pulmonary phthisis holds equally for other organisms. When the body is making a satisfactory immunizing response, the index may be maintained at a constant high level and above normal; when auto-inoculations are occurring, the index will be fluctuating; while in acute cases, where the immunizing machinery is in default, the index will be low.

(b) *In Chronic Cases*.—Here the index is almost uniformly low. Should, however, an auto-inoculation occur, then an immunizing response may be elicited, and the index be raised above normal.

Da Costa<sup>1</sup> studied the opsonic index towards *Staphylococcus aureus* in twenty-two cases of diabetes. Sixteen of these were cases of true diabetes mellitus, and all showed subnormal indices, varying between 0·34 and 0·72, the average being 0·62. The liability of diabetes to boils and carbuncles is thus explained. Four cases of diabetes insipidus had indices between 0·8 and 0·9, and two of transient glycosuria had indices of 0·8 and 0·9.

C. J. Shaw<sup>2</sup> determined the indices in fifteen insane patients towards the tubercle bacillus, *Bacillus coli com-*

<sup>1</sup> *American Journal of Medical Science*, July, 1907, p. 57.

<sup>2</sup> *Journal of Mental Science*, January, 1908, p. 57.

*munis*, staphylococcus, and *M. rheumaticus*. The number of cells observed was never more than fifty, and his figures do not appear very convincing, but he concluded that in the insane the index to the above organisms is generally lower than in the sane, and the amount of variation greater. From consideration of the indices he deduced that the acutely mentally affected are more liable to organismal infection than more chronic cases, but that the latter have less resisting power than the sane.

#### THE EFFECT OF MENSTRUATION UPON THE INDEX IN INFECTED CASES.<sup>1</sup>

An important point to remember in connection with a female infected by any organism is that menstruation produces a very marked lowering of the index to that organism—an effect which may begin a day or two before the period and persist for a day or two after ; the fall and rise, once initiated, move with great rapidity. It is, however, stated that in non-infected females there is a general depression of the opsonic index to all organisms ; this statement needs confirmation.

#### THE OPSONIC INDEX AS AN AID TO DIAGNOSIS.

1. *In Cases of Supposed Tuberculosis*.—It has been mentioned that the index to the tubercle bacillus of the sera of healthy subjects varies between 0·8 and 1·2. The important question now presents itself as to how we are to regard an index which does not lie within these limits. Does it mean that infection has already taken place, or merely that the person is predisposed to it ? That a low index always means the former of these alternatives is certainly not the case, as is shown

<sup>1</sup> French, *Practitioner*, July, 1906.

by the following instance : Dr. Eyre, while directing the work of the Commission on Mediterranean Fever in Malta, contracted the disease severely. After a short interval it was found that his index to the tubercle bacillus, which was known to be normal before his departure from England, was below 0·4. It remained at this low level for several weeks, and only slowly returned to normal. There never was any evidence soever of his having been infected by tubercle. A similar effect was also noticed in the case of Dr. C. Pryce Jones after contracting Malta fever.

It is probable that a low index predisposes to and so precedes infection, and is due either to an acquired or hereditary inability to elaborate the chemical protective substances of the body. It is possible that a fall in these bacteriotropic substances, which is local and not general, will suffice to determine infection in certain cases. As we have seen, a low index is the rule in chronic localized infection ; and in any case of supposed tuberculosis where a low index is found, especially in the instance of a patient not coming from tubercular stock, and where clinical appearances are compatible with such a diagnosis, tuberculosis is highly probable. It must not be forgotten that a depression of index may persist for a long time after an infection is supposed to have been cured. Thus, in fourteen picked cases of sanatorium 'cures' of phthisis in its early stages, Bulloch<sup>1</sup> found indices varying between 0·4 and 0·86. Lawson and Stewart<sup>2</sup> examined the indices of twenty-five such cases. In five of these it was found to lie between 1·1 and 0·9 ; in the other twenty it was 0·8 or under. Consideration of these results, taken in

<sup>1</sup> *Lancet*, December 2, 1905, p. 1603.

<sup>2</sup> *Ibid.*, December 9, 1905, p. 1683.



conjunction with the extreme frequency with which indications of healed tuberculosis, either of bronchial glands or lungs, are found in autopsies upon those never recognized as tubercular subjects while alive, tends strongly to support West's view that all cases in which low indices, not explicable by such considerations as were noted in Chapter I., are found are instances either of cured or active tuberculosis. On the other hand, an abnormally high index—1·3 or over—is probably almost always a sign of active infection.

Reliance should not, however, be placed upon a single determination of the index ; two at least are always advisable. Should these not agree, then a series should be done before a definite conclusion is arrived at or estimation made of the amount of thermostable opsonin (see p. 6). Continual variations certainly indicate active infection and a succession of auto-inoculations. *Per contra*, the non-occurrence of a high or fluctuating index in patients acutely ill is very strong evidence against a diagnosis of tuberculosis, and lends support to such alternative diagnoses as malignant disease of the lung, chronic bronchitis and emphysema, bronchiectasis, general debility, or gonorrhœal arthritis.

An abnormal index will assist in discriminating such conditions as—

Tuberculous kidney from malignant kidney or renal calculus.  
Addison's disease from pernicious anæmia.

Tubercular peritonitis from malignant peritonitis.

„ laryngitis from malignant laryngitis.

„ pleurisy from malignant and other forms of pleurisy.

„ joints from syphilitic and gonorrhœal joints.

„ adenitis from Hodgkin's disease.

„ endocarditis from fungating and other forms of endocarditis.

„ keratitis and iritis from syphilitic and rheumatic.

- Tubercular epididymorchitis from syphilitic, adenomatous, or malignant forms.  
 „ cystitis from that due to calculi, tumours, enlarged prostate, etc.  
 „ salpingitis from gonorrhœal.  
 „ ovary from malignant or cystic.  
 „ endometritis from malignant, etc.  
 Lupus from syphilis or rodent ulcer.

2. *In Other Cases.*—Opsonic index determinations have been shown in numerous instances to be of the utmost value in the investigation of infections of doubtful nature. Thus, Houston and Rankin<sup>1</sup> demonstrated their great value in the diagnosis of suspected cases of cerebro-spinal meningitis, in which the index is always high, and in the differentiation of the true form of this disease from posterior basic meningitis.

In three cases of suspected non-specific urethritis the author has been enabled to make a definite diagnosis, and to exclude the gonococcus—rightly, as subsequent events proved. The differentiation of an acutely rheumatic or gonococcal joint from one infected by other pyogenic cocci is greatly facilitated, and the correct diagnosis of the infecting agent in septicæmic cases where no organisms can be isolated from the blood rendered possible. A study of the opsonic index in typhoid fever may well prove especially productive of valuable results, and enable a diagnosis to be established earlier than is now possible by any other means.

The careful study of Wright and his co-workers<sup>2</sup> upon the artificial production of auto-inoculations has rendered available a method of extreme value for determining whether an ineffective focus has completely healed or merely lies dormant.

<sup>1</sup> *British Medical Journal*, November 16, 1907.

<sup>2</sup> *Lancet*, vol. ii., 1907, p. 1217.

### EFFECT UPON THE OPSONIC INDEX OF INJECTION OF A BACTERIAL VACCINE.

The statement is usually made that in a healthy person the result of the injection of a bacterial vaccine upon the index to that organism is very slight. Any subsequent depression of the index is stated to be of a very temporary character and of only small extent, while the rise of index which follows the return to the normal is also of a limited and slight character. This statement is substantially true for the tubercle bacillus, but does not hold equally for other organisms, as the following experiments will show :

*Experiment I.*—An injection of 250,000,000 dead organisms of the *Bacillus septus* was given to a healthy person not infected by that organism, and samples of blood taken on injection and after intervals of three, six, twelve, eighteen, twenty-four, thirty-six, forty-eight, seventy-two, and ninety-six hours. The comparative opsonizing powers of the various sera towards the *Bacillus septus* were then determined in the usual manner, with the following results :

TABLE VI.

Serum	1 on injection	882 bacilli in 200 cells.	Index = 1·00
„	2 after 3 hours	544	0·62
„	3 „ 6 „	885	1·00
„	4 „ 12 „	804	0·91
„	5 „ 18 „	949	1·08
„	6 „ 24 „	1,096	1·25
„	7 „ 36 „	1,000	1·15
„	8 „ 48 „	1,044	1·20
„	9 „ 72 „	1,220	1·38
„	10 „ 96 „	1,248	1·39

*Experiment II.*—An exactly similar experiment was done upon a second healthy individual, 350,000,000 organisms of the *Micrococcus catarrhalis* being injected, with the following result :

TABLE VII.

Serum	1	before injection	942 cocci in 200 cells.	Index = 1·00
„	2	after 3 hours	722 „ „ „	0·77
„	3	„ 6 „	580 „ „ „	0·62
„	4	„ 9 „	825 „ „ „	0·88
„	5	„ 15 „	1,125 „ „ „	1·20
„	6	„ 22 „	1,080 „ „ „	1·15
„	7	„ 28 „	1,131 „ „ „	1·20

In both of these experiments we see a very pronounced depression indeed produced in the index, in each case to the extent of 0·4. The duration of this depression was short, it is true, but no shorter than that obtained in a similar experiment upon an infected person, as is seen in—

*Experiment III.*—From the tracheal mucus and nasal secretion of an individual who had been suffering for a fortnight with a very bad tracheal cough a practically pure culture of the *Micrococcus catarrhalis* was isolated, and the index found to be 0·56. An injection of 250,000,000 organisms was given, and the effect upon the index determined as follows, the index prior to injection being called unity :

TABLE VIII.

Serum	1	before injection	140 cocci in 100 cells.	Index = 1·00
„	2	4 hours after	90 „ „ „	0·64
„	3	8 „ „	220 „ „ „	1·57
„	4	12 „ „	280 „ „ „	2·00
„	5	15 „ „	312 „ „ „	2·23
„	6	18 „ „	325 „ „ „	2·32

The chief difference is the much more pronounced subsequent elevation of index. To the depression of the index the term 'negative phase' was given by Wright, while the subsequent rise he called the 'positive phase.' The negative phase thus comprises the interval when the index is falling, and also that when it is rising until the level at which it stood prior to injection is again attained. The full rise having been attained, the crest of the positive phase may be said to have been reached, as at seventy-two hours in Experiment I. and at fifteen hours in Experiment II. (*supra*). The index remains practically steady at this elevated level for a time, which varies in different individuals and for different organisms—it may be for hours, days, or even weeks. This may be termed the 'positive phase plateau.' It then begins to fall, and falls with a rapidity which also differs in different cases.

During the period of falling in the negative phase an infected patient may present marked clinical features. For instance, in cases of acne a fresh crop of pustules usually appears; in cases of cold the cold gets worse; in tubercular cases the patient may feel restless and ill or experience increased pain in a joint. The temperature, pulse, or respiration are, according to the dosage, also more or less affected. Very soon indeed after the inception of the rise, even before the index has reached the level at which it stood prior to injection, the patient may begin to improve and declare himself to feel better.

A very marked instance of this was afforded in the case of a severe gonococcal conjunctivitis, in which the pain, discharge, and chemosis all diminished two days before the index had reached the level at which it originally stood. *The* factor in improvement would, therefore, appear to be a 'rising' index.

It must, however, be mentioned that the above is not a complete description of all that occurs or may occur after the injection of a bacterial vaccine, and other variations may be introduced by modifications in the dosage.

Thus, it is probable that with a medium dose the first effect is a very slight and very transient fall indeed of the index, due to the immediate combination of the opsonin at the site of injection with anti-opsonin present in the vaccine. To this the body makes reaction by formation of fresh opsonin, with the result that there ensues a short period of slightly raised index, and it would appear that the improvement sometimes seen during the supposed 'negative' phase is in reality due to this initial temporary rise.

After this oscillation the true negative phase begins, to be succeeded by the positive phase, though fresh oscillations may occur at any period.

With minimum doses of a vaccine, on the other hand, all oscillations and the negative phase itself may be elided, and injection be followed by an immediate rise, limited alike in extent and duration.

Buxton<sup>1</sup> has demonstrated the following important difference in the behaviour of a normal and of an immunized animal to infection : In the former phagocytosis is weak and extracellular bacteriolysis strong ; as a result of these two factors extracellular destruction of bacteria is great, and the consequent liberation into the circulation of the bacterial endotoxins is great, whereby severe reaction upon certain cells, such as those of the nerve centres, is produced. In the 'immunized' animal, on the contrary, phagocytosis is strong and extracellular

<sup>1</sup> *British Medical Journal*, November 6, 1907, p. 1421.



bacteriolysis weak. The destruction of the bacteria, therefore, chiefly occurs intracellularly, and the endotoxins are destroyed before these can enter the general circulation, and so reach the nerve centres.

#### DURATION OF NEGATIVE PHASE IN PHTHISIS.

Lawson and Stewart<sup>1</sup> investigated the duration of the negative phase in 120 cases of phthisis. Their results were as follows :

No negative phase in 15 cases.

Persistent negative phase in 21 cases.

Negative phase lasting—

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days.  
in 14, 12, 10, 5, 7, 4, 10, 4, 4, 3, 4, 1, 1, 5 cases.

Total : 84 cases.

It would thus appear that in 41 per cent. of the cases the negative phase lasted over a week. But, as has been already mentioned, the duration of negative phase depends very largely indeed upon dosage : the smaller the dosage the shorter the negative phase ; thus, with doses of 0·00001 c.c. to 0·00003 c.c. of tuberculin (T.R.) the average duration is about three days. Upon the important questions as to the interval occupied in various cases in attaining the crest of the positive phase and the duration of the plateau published information is lacking.

The significance of a persistent negative phase after a first injection of tuberculin is great. It may mean that the case is one altogether unsuited to this course of treatment on account of the immunizing machinery having altogether broken down ; it may mean that the initial dose was much too large, in which case it is unnecessary

<sup>1</sup> *Lancet*, December 9, 1905, p. 1682.



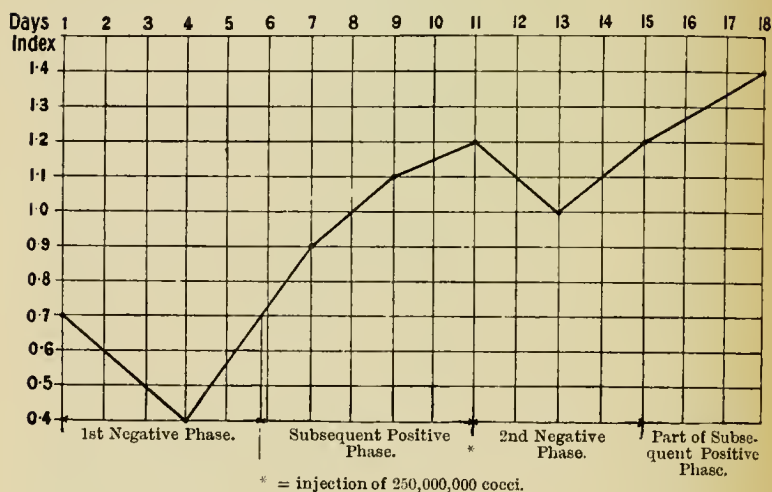
to wait any longer than until all constitutional symptoms have disappeared before reinoculating at this level with a much diminished dose ; or it may be merely a peculiar phenomenon that the author has several times experienced, especially in chronic gonorrhœal cases. As subsequent events showed, the immunizing machinery was far from exhausted, nor was the dose too large, yet the index fell markedly, and there it remained for weeks at a new low level. The repetition of the original dose resulted in a perfectly satisfactory response, as did all subsequent injections. In these instances, then, it is probably good practice, except in obviously bad cases, to repeat the original dose a second time. A further fall and persistence of the negative phase would be warning to wait a few weeks, and then begin again with a dose only a third or quarter as great as that previously employed. Yet, again, in cases which have been doing well and had several injections, the usual dose will produce an unexpectedly long negative phase, lasting for three, four, or five weeks, although the patient apparently continues to improve.

#### THE CUMULATION OF NEGATIVE AND OF POSITIVE PHASES.

A second injection during a negative phase will result in further depression of the index to yet a lower level—that is, one negative phase may be superimposed upon another. The same holds true for positive phases, and this production of cumulated positive phases is the aim in vaccine treatment when indicial guide is employed. It still remains true, however, that the first result of an injection is to produce a negative phase, so that a slight lowering of the raised index at first results, to be followed

by a further rise. This cumulation of positive phases may be thus shown diagrammatically (Chart IX.) :

CHART IX.



It is generally held that in tuberculous infections a cumulation of positive phases cannot be produced, and it is advised that the good results of one injection should be allowed to take full effect before again inoculating. This means, as a rule, an interval of ten to fourteen days being allowed. In other infections the aim is to give a fresh inoculation while the positive phase is at its height, rather than when the index has begun to fall.

#### THE OPSONIC INDEX AS A GUIDE IN THE REGULATION OF DOSAGE AND TIME OF INOCULATION.

That doses of appropriate magnitude be employed is of importance secondary not even to proper spacing of the several inoculations. Experience has shown that

the proper initial dose varies considerably for different organisms and to a less extent for different persons. The average initial dose for each organism is given later. Let us suppose that this dose has been given in a certain instance, the index prior to inoculation having been found to be subnormal. A fresh determination of the index is made twenty-four hours after injection, and again seven or ten days later.

The various possible results and the deductions therefrom may be thus displayed schematically :

Index 24 Hours after Injection.	Index 7 or 10 Days Later.	Deduction.
Slight fall.	Further fall.	Dose too large.
Slight rise.	But little altered.	Dose too small.
Slight fall or rise.	Marked rise.	Dose correct.

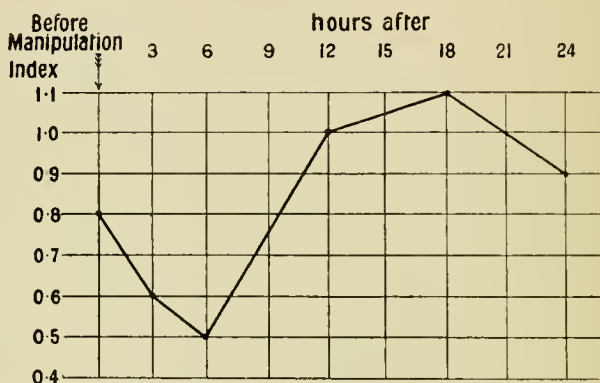
It will be found, as treatment progresses, that gradually increasing doses, often at shorter intervals, have to be employed to produce any marked effect upon the index. Thus, in staphylococcal cases an initial dose of 100,000,000 organisms may have finally to be increased even to 5,000,000,000 before a cure is effected. So long as a certain dose produces an adequate response, increase of it is not advisable, but so soon as this result is not achieved the indication for doubling the dose is present. The approaching termination of infection is indicated when these large doses finally fail to produce a rise of more than one or two decimal points in the index, which assumes a level at unity or slightly above it. One or two more large doses are then to be followed by diminished doses at increasing intervals.

### SPECIAL METHODS OF EMPLOYING THE OPSONIC INDEX IN DIAGNOSIS.

The first method depends upon the artificial production of an auto-inoculation, and is especially applicable to tuberculosis and other diseases of joints. The index is taken ; passive movement of the joint is then performed by the surgeon, and fresh determinations of the index done after three, six, twelve, and twenty-four hours. Should infection not be present, minimal variations in the index will be found ; but should it be present, the production of definite negative and positive phases will be evidenced. The negative phase may be fully produced within three hours or may be absent.

The adjoined chart shows the effect produced in a case of doubtful tuberculosis of the hip in a child six years old :

CHART X.



Ample confirmation of the diagnosis was thus secured.

The second method is of wider applicability, and depends upon the effect produced upon the index by the injection of a vaccine (see p. 64). A small dose of T.R.

(0·00002 to 0·00004 c.c.) is given, and the index estimated daily.

In healthy subjects the negative phase, if present at all, is of very slight amplitude and short duration, lasting, as a rule, for only a few hours. The positive phase resembles the negative, and the limit of fall and rise in the index rarely exceeds 0·2 or 0·3. Lawson and Stewart<sup>1</sup> found no negative phase, but a rise in one instance from 1·0 to 2·1 within a few hours. In infection, on the contrary, a much more pronounced fall and rise are, as a rule, obtained; the negative phase may last for several days, and the crest of the positive phase be not attained for one or two weeks. Inasmuch as occasionally no negative phase is produced in cases undoubtedly tubercular, failure to obtain a negative phase does not entirely put the diagnosis of tubercle out of consideration. A third method is the employment of the original tuberculin in doses insufficient to produce the acute disturbances originally obtained. Dodds<sup>2</sup> reported the effect of injections of T.O. in doses containing 1 milligramme of solid substance upon the opsonic index in five doubtful cases of phthisis, and in one certain case exhibiting bacilli in the sputum. In this latter instance the index before injection was 0·7; twelve hours after injection of 0·5 milligramme of T.O. the index=1·1; upon the fifth day it had fallen to 0·9. Of the other five cases, in four the index was normal, and remained so after injection of 1 milligramme of T.O. Of these, three were cases of old pneumococcal pleurisy, with chronic cough for months; the fourth had a chronic cough. In the fifth case the

<sup>1</sup> Edin. Med.-Chir. Trans., November 1, 1905. Proc. Royal Med.-Chir. Soc., November 28, 1905.

<sup>2</sup> *British Medical Journal*, July 7, 1906, p. 22.

index fell from 1·0 to 0·7, and rose next day to 1·4. He had a phthisical family history, and had had pleurisy six years previously, with persistent subsequent cough; his sputum was streaked with blood, and occasional night sweats were present. In this case the diagnosis of phthisis was held to be confirmed.

A fourth method depends upon the fact, already noted, that in females infected by an organism the onset of menstruation initiates a very marked fall in the index towards that organism; the cessation, as pronounced a rise. If, therefore, a female be suspected of tuberculosis, determinations of the index a couple of days before the onset, towards the end of the period, and two or three days after, should reveal marked negative and positive phases.

In two cases of severe recurrent episcleritis which is by some eye pathologists considered to be of tubercular origin, I utilized this method. In the first the tuberculo-opsionic index before menstruation was 0·96, and during menstruation 0·93; tuberculin injections were therefore not advised.

In the second case it was 1·26 before menstruation, and 1·28 during the period. No other clinical evidence of tubercle could be found, and, as before, despite the somewhat high index, tuberculin injections were held to offer slight chance of improvement. As, however, the patient came from tubercular stock, and wished to avail herself of every chance, four injections were given at three weekly intervals, but without producing the slightest improvement.

A fifth method, whereby the presence of a suspected infection can be established, has been devised by Peel Ritchie,<sup>1</sup> and should prove especially useful in cases

<sup>1</sup> *Lancet*, November 16, 1907, p. 1419.



where an index upon the border-line of the normal—say 0·8—has been obtained by the usual method. If the serum of an infected individual be compared with a normal serum, not only undiluted, but also when diluted with five volumes of normal saline, it will be found that the index is lower for the diluted than for the undiluted sample. But if the two sera be treated with a thick suspension of a bacterium other than that with which the individual is infected, and the index towards the infecting organism be determined for the diluted and undiluted sera, a different result is obtained; the serum of the infected individual will now show a much higher relative value than before. Thus the serum of a certain tuberculous person was compared with that of a healthy one and found to have an index of 0·73; both sera were then diluted five times with normal saline, and the index found to be 0·53. The phagocytic power towards the tubercle bacillus was then redetermined for the diluted sera, a considerable amount of a culture of *Bacillus coli communis* being also added to the phagocytic mixture. The tuberculo-opsonic index of the diluted serum of the infected individual was now found to be 1·31.

This depends upon the fact, as we have seen, that in the blood of an infected individual opsonin specific against the infecting organism is elaborated, and it is the amount of specific opsonin which is alone estimated by this latter procedure.

For tubercle estimations Ritchie advises the addition of *Bacillus coli communis* to absorb the non-specific opsonin, and for other estimations absorption with the aid of tubercle bacilli. The relative rise is rarely less than 0·2, and is often very striking. Ritchie employed this method in 150 cases of varied infections with striking



success. Failure occurred in only eleven cases, which were all tubercular ; the explanation of this will be given later (see Chapter XI.).

#### THE OPSONIC INDEX AS AN AID TO PROGNOSIS.

1. *In Pulmonary Phthisis.*—It is beyond question that the cases of pulmonary phthisis which do worst are the pyrexial ones, and, as we have seen, these exhibit violent fluctuations in the index. Rest in bed steadies the temperature and opsonic index alike. The level taken up by the latter varies considerably in different cases, and sufficient evidence is not yet forthcoming to enable a definite opinion to be given as to the import of a steady high or low index.

Taking into consideration the facts that chronicity is always accompanied by a low index, and that the aim of therapeutic injections is to raise the index to or above unity, it would appear rational to assume that those cases will do best which settle down to a steady index of 1 or over, while those that settle down to an index below 1 will go on to chronicity. Meakin and Wheeler support this view. They find that the case with an index much below 1 is the case that becomes chronic, that recovers to a certain extent, but can only maintain that degree of recovery while living under sanatorium treatment ; that the case, on the other hand, which during treatment shows a steady index of 1 or over is the one which makes a complete recovery if favourable conditions are maintained for a sufficient time. They lay especial stress on the statement that it is only to patients actually undergoing sanatorium treatment that this opinion applies.

Lawson and Stewart<sup>1</sup> took the indices of twenty-five cases of sanatorium 'cures.' In five of these it was found to be between 1·1 and 0·9; in the other twenty it was 0·8 or under.

In thirty other similar cases fourteen had indices between 0·5 and 0·9, and of these thirty cases twenty-nine had been carrying on their usual occupations, in most instances in towns, for periods ranging from six months to four and a half years, and enjoying perfect health.

As to the liability of cases with low indices to relapse, nothing definite is at present known, but authorities agree upon the distinct advisability of artificially raising to unity or over the indices in all such cases. The cases which seem to profit most when tuberculin injections are added to the other therapeutic measures adopted at sanatoria appear to be those with initially low indices, although improvement is also noticeable in those with indices above unity.

2. *In Other Tubercular Affections—Lupus.*—Bulloch's experience is that the cases which do best with Finsen light are those with indices either beyond or within the normal limits; those with indices below 0·8 do worst, whereas, *per contra*, those cases which profit most from tuberculin injections are those of the latter class. Wright finds that in those varieties of lupus where the infected skin is dry and scaly, so-called lupus psoriasis, tuberculin is of little avail; while in suppurating lupus, where mixed infection by the *Staphylococcus albus* is present, good results can often only be achieved by a simultaneous attack upon the secondary infection.

As regards other tubercular affections, such as those of

<sup>1</sup> *Lancet*, December 9, 1905, p. 1683.

glands, peritoneum, joints, kidneys, and bladder, no definite rules can be laid down beyond the general statement that, if tuberculin injections are not to be given, the cases that have a steadily high index do best, while those with fluctuating indices do badly, and those with subnormal indices show little tendency to recover. These last show the relatively greatest improvement under a course of tuberculin, but as experience increases it becomes more and more difficult to draw the line between suitable and unsuitable cases for such treatment, for some cases which have seemed the most hopeless have yet done well. In four cases of tubercular peritonitis which relapsed, White found subnormal indices ; in one which recovered, an index above normal.

3. *In Other Infections.*—No general rule whatever can here be laid down. A low index denotes lack of immunizing response, and therefore chronicity or complete ultimate failure of the protective mechanism ; a high index obviously denotes an attempt at adequate immunizing response. The attempt may succeed, or it may fail, either because it is inadequate or because the other protective mechanism breaks down. That death is often preceded by an abnormally high index has mystified many ; in reality there is no difficulty in understanding it at all. It is then a last supreme effort on the part of the body to overcome the infection ; into what may be its most powerful line of defence it hurls up all its reserve supports, but in vain. The other protective mechanisms do not suffice ; the vanguard is strong enough, but the flanks are weak and the rear unguarded. For instance, in a case of septicæmia the opsonin may be adequate—perhaps more than adequate—but what avail is this if the myocardium has been hopelessly weakened

by toxin ? Of what use, again, is an index of 15 in cerebro-spinal meningitis, when the cerebro-spinal fluid has an index of zero and all the cerebral centres are overloaded with toxin ? Yet the body will keep up the fight to the bitter end, and in a last effort perhaps raise the index to 30.

#### ELEVATION OF THE OPSONIC INDEX NOT THE SOLE NECESSITY.

Wright has been at especial pains to point out that the successful combat of bacterial invasion does not depend upon elevation of the opsonic index alone. Increase in the bacteriotropic substances of the blood having been secured, it still remains to insure that these be brought in sufficient amount to the point of attack. Experiment has shown that the fluid portion of pus may be entirely free from opsonin, while the amount of the latter in the serous exudates in pathological conditions of the peritoneum, meninges, pleura, and pericardium may be very greatly diminished. It therefore becomes necessary to insure the removal of the fluid poor in antibacterial substances, and its partial replacement by lymph rich in such substances. This end is secured in various ways, as by opening a fluctuating abscess, doing a laparotomy upon a tubercular peritonitis, or tapping an empyema. Other cases there are, such as more or less non-discharging sinuses, where dense granulation tissue and deposits of fibrin prevent free access of lymph, and brawny swellings where the same result is brought about by blockage of the lymphatics. The former of these conditions Wright meets by the introduction into the sinus of a solution of 0.5 per cent. citrate of soda and 5 per cent. sodium

chloride, the former decalcifying the lymph, and so preventing its coagulation, the latter by osmosis causing transudation of fluid from the vessels.

The surgeon has been wont to secure a similar result by scraping and the application of caustics. Brawny swellings are to be freely incised, and the coagulability of the lymph diminished by three-hourly doses of 60 grains of sodium citrate.

#### THE VALUE OF THE OPSONIC INDEX AS A GUIDE IN VACCINE THERAPEUTICS.

To summarize the preceding pages, it would appear that there is in the blood a protective substance to which the name 'opsonin' has been applied, and for its measurement a procedure has been devised whereby the so-called 'opsonic index' is determined. The actual procedure is in itself capable of being conducted with approximate and sufficient accuracy. It now becomes necessary to consider (1) whether the opsonic index accurately determined really measures anything of importance in the immunizing processes of the body against bacterial infection, and (2) whether use may be made of it as a guide in the production of immunity artificially brought about by the introduction into the system of killed cultures of the infecting organism.

Let us first consider what the opsonic index really measures in the case of a tuberculous infection.

Upon each side of the equation there are introduced the following factors : (1) Leucocytes, (2) bacilli, (3) serum ; and each one of these may have nothing whatever to do with the unknown.

(1) Consider first the leucocytes. These as often as not

are derived neither from the patient nor control. It is perfectly true that the opsonic index does not purport to take inherent variation in phagocytic power of the leucocytes into account, but as the object is to determine the relative power of the patient's blood to cope with bacterial invaders, it should do so.

A still more pregnant objection to the use of leucocytes where an estimation of the tuberculo-opsonic index is aimed at is that leucocytes are possibly not concerned as phagocytes in resisting infection by tubercle bacilli. The statement is made—I believe with truth—that no one has ever seen a tubercle bacillus inside a polymorphonuclear leucocyte derived from a tuberculous focus. The rôle is assigned to giant and lymphoid cells.

(2) As regards the bacilli, these have been derived from an alien source, and may not even be of the same strain as those producing the infection. The ease with which long-cultured strains of bacteria phagocytose is proverbial.

(3) As regards the serum, we are dealing entirely with an artefact. That serum and plasma contain the same amount of opsonin is almost certainly not the case, and as to whether the proportionate variation is constant we know not at all. In brief, more or less accurate measure has been made of a coefficient which has little or nothing to do with the one whose value we wish to determine.

But it may be urged that the hæmophagocytic index, in the determination of which the patient's own leucocytes are employed and plasma is substituted for serum, agrees reasonably well with the opsonic index, and that in other bacterial infections the patient's own organisms are usually employed for the preparation of the emulsion. Granting this, it yet remains that only in those cases where phagocytosis is the chief defensive mechanism



against the given bacterium is a measurement made of anything which plays the predominant part in the production of immunity. To assume that in measuring the less we are at the same time measuring the greater is entirely indefensible until a better knowledge of the part played by each of the defensive mechanisms of the body has proved the validity of this deduction.

Upon the other hand, the difficulties liable to be encountered by those who would undertake the conduct of therapeutical immunization without the guidance of the opsonic index are so clearly outlined by Sir A. E. Wright, in his article in the *Practitioner* for May, 1908, that I cannot do better than make the following extract :

‘ The suggestion has been proffered by many that the clinical symptoms of the patient will furnish the immunizator with a guide by which he may regulate his procedure, but to this suggestion many objections may be taken. For instance—

‘ 1. Even in the case of localized infections, where objective and other signs are freely presented, many difficulties of interpretation may be present. Thus, in lupus secondarily infected by streptococci incidental exacerbations of the secondary infection may completely mask amelioration in the tubercular infection ; and this is equally true of other mixed infections. Again, variations in the size of tubercular glands, and perhaps changes in the amount of effusion into tubercular joints, may occur, independently of any progress or regress of the infection, directly as the result of changes in the coagulability or viscosity of the blood.

‘ 2. The clinical symptoms, even when conveying accurate information with respect to the conditions obtaining in the focus of infection, may suggest quite a wrong



picture of the conditions obtaining in the circulating blood.

‘ 3. The fact that the patient’s general condition remains undisturbed does not warrant us in assuming that the antibacterial potency of the blood is not undergoing momentous fluctuations under the influence of spontaneous auto-inoculations such as the immunizator should take into account in regulating his dosage and in interspersing his inoculations.

‘ 4. Many conditions there are of a strictly localized infection, where the conditions are unfavourable to the observation of changes in the condition of the focus of infection, such as the majority of cases of tubercular adenitis, arthritis, lupus, and phthisis. Of course, the clinical symptoms would ultimately inform as to the results of the course of vaccine therapy, but this information arrives too tardily to be of service. The necessity is obvious of determining, from time to time, what the scheme of dosage is achieving for good or ill.

‘ 5. In acute febrile conditions, while it is true that the rule is to find an inverse relation of temperature to antibacterial potency, it is certainly not the invariable rule. It is notorious that excessive intoxication may condition a fall in temperature, and it is conceivable that a rise in temperature may sometimes be associated with efficient immunizing response.

‘ 6. Finally, there are cases where all local and general symptoms are in abeyance, or have returned to the condition which prevailed previous to inoculation. Here we are face to face with the difficulty as to whether the patient is now immune, whether the infection has been eradicated or still persists.’

What, then, is to be done? That upon the deter-

mination of the opsonic index and the control thereby of a course of immunization the whole superstructure of vaccine therapy has been built, and built with a success which is remarkable, must be conceded. Are we, then, to cast aside as useless the guide which has proved so serviceable in the past, and employ no other ? and is the alternative so bad as would appear from these words of Sir Almroth Wright ? Before endeavouring to furnish the answer to these questions, it would perhaps be well to consider seriatim these objections of Sir Almroth Wright.

1. To this it may be rejoined that in cases of lupus secondarily infected by streptococci the wiser procedure is first to deal with the streptococci, which for the time being are the more important invaders. The tuberculin treatment of lupus has not met with such conspicuous success that much will be lost by directing the main attention to the streptococci. In the case of other mixed infections, careful microscopy of the secretion will usually afford a fair index as to how the infection is progressing ; where there is no secretion, other clinical signs and symptoms may be consulted. Again, the size of tuberculous glands or joints is not the only indication of progress or regress, and swelling due to changes in the viscosity or coagulability of the blood is both rare and transitory. Other than local conditions may well produce a variation of a decimal point or two in the opsonic index, and if the indicial guide is being employed, this variation will be interpreted as being due to local changes which have never occurred.

2. To this it may be replied that improvement at the focus of infection is the main consideration ; that if this be improving there is little to fear elsewhere. Upon the other hand, it would be quite wrong practice to fail to

immunize a patient suffering from, say, gonococcal conjunctivitis, whose anterior chamber was filling with pus and cornea threatening perforation, because the gonococcal index was high ; for so, as a rule, it is in these cases.

3. Where the antibacterial potency of the blood is undergoing such momentous fluctuations as Sir Almroth Wright pictures, it must surely be extremely rare that to the discerning eye no warning alteration in pulse, temperature, respiration, or general condition, is apparent. In such a case the first doubts should be cast upon what the laboratory method is really measuring, upon how this measurement has been performed, and upon its significance.

4. The instances here given are peculiarly ill-adapted to prove the point, for above all, in infections by the tubercle bacillus, has it been shown that the procedure employed in the determination of the opsonic index is ill-adapted for estimating the powers of defence at the command of the body. In lupus tuberculin treatment has met with scanty success, and in phthisis there are many other guides at our disposal.

5. It has not yet been shown, in acute febrile conditions where the intoxication is so excessive as to condition a fall in temperature, that the opsonic index affords any efficient guide to immunization. Such cases often show a greatly raised index, signal to the immunizator that adequate response is being made to the auto-inoculations, yet this we know well not to be the case.

In the four worst cases of acute febrile conditions with which I have had to deal (for two of which see *Lancet*, September 13, 1909), the guide of opsonic index was dispensed with, and that of pulse, temperature, and general condition, substituted with complete success.

6. In this instance the objection is a real one, and index determinations will often shed light upon what is dark and obscure.

It must not be thought from the above that I consider the opsonic index as entirely useless as a guide to the immunizing processes going on in the body. In such infections as those due to the staphylococcus and pneumococcus, where the chief defensive mechanism appears to depend upon phagocytosis, the opsonic index probably affords a sufficiently accurate guide ; but, unfortunately, these are just the cases where local signs and general symptoms equally well suffice.

In short, as a means of diagnosis the opsonic index is sometimes—nay, often—invaluable, and can at present be replaced by nothing else, though even here the various ophthalmic and cutaneous reactions threaten soon to take its place. As a guide in the artificial production of immunity it is sometimes misleading, but much more often useful, just as are pulse, temperature, respiration, and local and general conditions. Except for its aid, vaccine therapy would never have occupied the place it does to-day, but obstinate adherence to it will only tend to impede the march of progress. In subsequent pages frequent reference will be made to the results of opsonic index determinations, mainly in order to show by what steps the truth has been elucidated and our present knowledge attained. What substitutes for its guidance are to be employed will be found there also.

## CHAPTER V

### INFECTIONS BY THE STAPHYLOCOCCUS AND THE ACNE BACILLUS

THE *Staphylococcus albus* and *aureus* may be the cause of inflammatory and suppurative processes in various parts of the body. Among acute forms of infection may be instanced Suppurative Periostitis and Osteomyelitis, Ulcerative Endocarditis, Pleurisy, Peritonitis and Meningitis, Carbuncle and Furuncle, Endometritis, and various Pyæmic conditions ; among its chronic manifestations are Acne, Ulcers, and Sycosis. It may also secondarily infect cases due to the Tubercle Bacillus, *Bacillus coli communis*, *Bacillus typhosus*, Streptococcus, and Gonococcus.

The toxins produced by this organism appear to be (1) lysins for the red blood-corpuscles (*i.e.*, a hæmolysin), and for the leucocytic cells (*i.e.*, a leucocidin) ; (2) an endotoxin. Against the hæmolysin and leucocidin the human body naturally elaborates antibodies, which are not increased by the administration of a vaccine. Ordinary methods fail to demonstrate any bactericidal action either in normal or immune sera. Andrewes and Gordon, and also Meakins,<sup>1</sup> by special methods have, however, shown that a lysin for the staphylococcus does occur in small quantities in normal sera, and to a greater extent in immune sera. Immunity against this organism thus almost entirely depends upon phagocytosis, which is

<sup>1</sup> *Journal of Experimental Medicine*, January, 1909, p. 100.

capable of great activity, and is increased by the administration of a vaccine. The opsonic index affords a comparatively reliable measure of the degree of immunity possessed by the individual. The necessity for its determination is, however, rendered much the less pressing from the fact that clinical observation of the lesions is usually practicable, or, where this is not the case, pulse, temperature, and general condition, suffice.

#### ACNE.

In 'The Opsonic Method of Treatment' (1907), p. 99, and in 'Vaccine Therapy' (1908), p. 153, appears the following: 'A small percentage of cases remain entirely obdurate, while others relapse after apparent cure.' And again, on p. 151 of the latter: 'The type of case which yields the least satisfactory results is that characterized by marked oily seborrhœa, abundant comedo, and few suppurative foci.' These were almost the only warning words that appeared in print of the failure of staphylococcal vaccine to cure all cases of so-called 'staphylocosis.' The recent work of Fleming<sup>1</sup> and of Gilchrist, confirmatory of earlier observations by Unna and by Sabouraud, affords ample explanation of these failures by the establishment of the fact that acne is in over 50 per cent. of the cases a double infection by the *Staphylococcus albus* and acne bacillus, and in a smaller percentage of cases due to the acne bacillus alone.

Inasmuch as special cultural methods have to be employed for the isolation of the acne bacillus, the necessity of careful microscopy of the secretion from several separate pustules, if possible of different clinical

<sup>1</sup> *Lancet*, April 10 and 17, 1909.



varieties, is now obvious, in order that determination may be made as to whether the infection is one due to the acne bacillus alone, the staphylococcus alone, or to both bacilli together. In the first instance a vaccine of the acne bacillus must, of course, be employed ; in the second instance one of the staphylococcus ; in the third both vaccines must be used together. The acutely pustular form is usually due to the *Staphylococcus albus* alone, the variety characterized by abundant comedo and marked oily seborrhœa often to the acne bacillus alone, but neither of these is by any means an invariable rule.

The index in acne produced by either organism or by both is invariably low to the infecting bacteria, ranging between 0·2 and 0·8. The control of dosage by means of the opsonic index is no longer necessary in this complaint ; treatment may well be conducted with the help merely of clinical observation. For adults an appropriate initial dose is 100,000,000 of the staphylococcus, or 5,000,000 of the acne bacillus, either separately or in combination, according as to whether the infection is a single or a double one. A slight negative phase, lasting for about two days, will result, during which there may appear an exacerbation of symptoms, such as a fresh crop of suppurative foci, which will, however, abort in a day or two. Cessation of improvement in the symptoms, or retrogression, as evidenced by the appearance of fresh pustules, is the signal for further injections. The usual interval necessary is between seven and fourteen days. Should no improvement or change have resulted, a dose of 250,000,000 staphylococci should be used upon the second occasion ; that of the acne bacillus should remain at 5,000,000. In the event of even the most temporary amelioration, dosage should remain as before.



So long as clinical improvement continues, there is no need for increased dosage; but when this ceases with each dosage, the succeeding one for the staphylococcus should be of double dimensions. Cure should not be despaired of until doses of 5,000,000,000 or even 10,000,000,000 have been attained; that of the acne bacillus should only in very exceptional cases exceed 20,000,000.

In one of the worst cases of acne which I have ever seen, doses of 1,750,000,000 staphylococci were given towards the end of treatment at fortnightly intervals, the index then remaining steadily between 0.9 and 1.2, when recovery was soon completed.

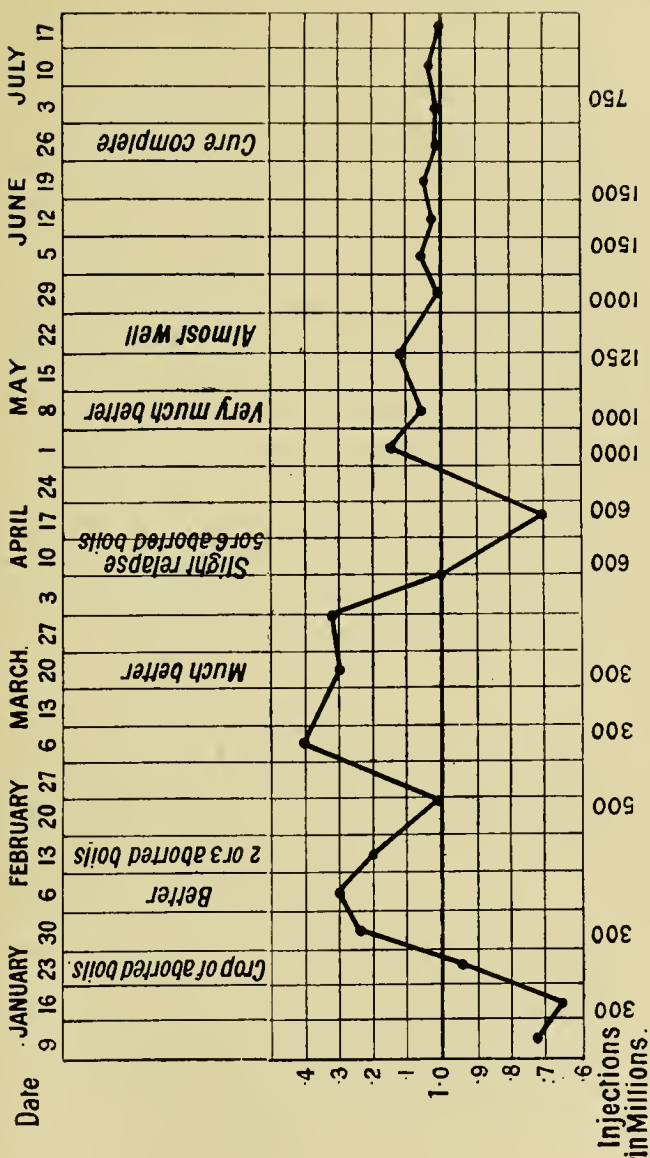
It is well to note that very slight cases may prove extremely obdurate, especially if the general health of the patient be good and the bowels require no regulation. In one such case—the mildest that I have had under my care—a very confident prognosis was given, with the result that in six months little progress had been made, and cure was only secured by the use of a dosage of 4,000,000,000 at ten-day intervals.

In the case of deep-seated foci which refuse to come to a head—acne indurata—hyperæmia by means of dry-cupping is a very useful adjuvant to the vaccine therapy.

The type of case which yields the least satisfactory results is that characterized by marked oily seborrhœa, abundant comedo, and few suppurative foci; these are usually pure infections by the acne bacillus. Especial attention, both local and general, will require to be paid to the seborrhœa, and the comedines should be systematically expressed.

Treatment must be persisted in even for six or eight months, until not only do fresh foci fail to appear, but even the old scars have begun to disappear. Diminished

CHART XI.  
VERY SEVERE ACNE OF FACE, NECK, SHOULDERS, AND BACK.



doses at prolonged intervals will in most cases complete the cure.

Since the recognition of the fact that the acne bacillus is often a complicating factor, the percentage of cases of acne which fail to respond to treatment, if persevered in with adequate doses, has been reduced almost to vanishing-point.

Fleming states that an autogenous vaccine of the acne bacillus is not necessary, and that a stock one gives equally good results. This needs, I think, a little qualification. It is by no means certain that there are not two varieties of the acne bacillus, a large and a small one. The advisability of using a polyvalent stock vaccine is therefore evident. As regards cases due to the staphylococcus, these as a rule progress very satisfactorily under a stock polyvalent vaccine, but none the less the best possible results are only to be expected when the autogenous vaccine is employed.

#### BOILS AND CARBUNCLES.

The results recorded by many observers in boils and carbuncles, of which the greater proportion are due to '*aureus*,' have been uniformly good. Thus, Whitfield<sup>1</sup> says: 'In all the cases of furunculosis which I have treated I have obtained complete, and up to the present durable, success.' Western:<sup>2</sup> 'Nine cases of furunculosis were treated with "*aureus*" vaccine, in every instance with completely satisfactory results. All the cases of carbuncle were ones which, in spite of incision, fomentations, and local antiseptics, showed no adequate attempt at repair or

<sup>1</sup> *Practitioner*, May, 1908, p. 698.

<sup>2</sup> *Lancet*, November 23, 1907, p. 1449.

healing, yet all made rapid recovery.' Hartwell and Lee<sup>1</sup> draw the following conclusions from their results in 100 cases of '*aureus*' infections: 'Treatment with vaccines is the most effectual treatment for boils and carbuncles. There is marked diminution in the pain and tenderness. After twenty-four hours there is a profuse discharge, which continues till the focus clears up. With boils about the face, the especial value of the treatment is the prevention of scarring. Although the vaccine treatment does not prevent recurrence, cases of chronic furunculosis can be absolutely controlled by occasional inoculation.' The initial dose is from 100,000,000 to 250,000,000.

#### SYCOSIS.

Various factors tend to render the successful treatment of this complaint more difficult than the preceding. The disease is a very chronic one, and results in seriously diminished powers of resistance in the skin, and irritation is kept up by local conditions, such as nasal discharge and dust; the organisms are, moreover, difficult of access for the immunizing lymph. For these reasons relapse is also liable to occur. Depilation by the 'X rays' or other means will considerably aid in the cure, but even then treatment may have to be very prolonged.

#### PERIOSTITIS AND OSTEOMYELITIS.

When staphylococci complicate a tubercular infection, as in psoas abscess, fistula '*in ano*,' joint and bone disease, etc., vaccine injections prove of the utmost value to the surgeon. Indeed, I have seen cases which, despite repeated operation, persistently refused to get well, clear

<sup>1</sup> *Boston Medical and Surgical Journal*, vol. cvii., No. 16, p. 523.

up as if by magic after two or three injections of staphylococcal vaccine as adjuvant to tuberculin. Simple acute infections invariably do well, but long-continued cases, complicated by lardaceous disease, are not nearly so hopeful.

Walters, Coombe, and Solly,<sup>1</sup> record a very satisfactory result in a case of severe facial carbuncle, with Ludwig's angina and parotitis, in a girl aged seventeen. She was semi-delirious, with a temperature of 103° F., and pulse 140. A dose of 50,000,000 stock mixed staphylococcus vaccine was followed, two and five days later respectively, by doses of 25,000,000 of autogenous *Staphylococcus aureus* vaccine, and fourteen days later by one of 50,000,000. Drachm doses of citric acid were also given to diminish the greatly increased coagulability of the blood which was present in this instance, and appears to be usually so in these rare cases.

#### SEPTICÆMIC AND PYÆMIC CASES.

During the first few years of the practice of vaccine therapy, the opinion prevailed that the especial scope for this method of treatment lay in the domain of chronic infections. This opinion must, I think, be abandoned, despite the fact that it is obviously an easier task to arrive at a just appreciation of results in cases which have resisted other methods of treatment—*i.e.*, which have become chronic—for it is quite impossible to say whether an acutely infected case would or would not have recovered had any given line of treatment not been followed. Sufficient has, however, now been done to incline me to believe that in acute infections will the

<sup>1</sup> *Lancet*, July 17; 1909, p. 145.

most striking results of vaccine treatment be obtained, and that more especially will this be found to be true in the case of septicæmias. During the past year I have had experience of three very bad such cases, due respectively to (1) the pneumococcus, (2) the typhoid bacillus, (3) *Staphylococcus aureus* and *Streptococcus conglomeratus*. Each made a complete recovery, and will be referred to in its proper place. The impression they have left upon my mind is that no acutely septicæmic case should die if subjected to vaccine treatment, provided that this has been begun sufficiently early. It matters little what the temperature be—even 105° to 106° F.—or if the patient be acutely delirious. The important prognostic points are : (1) The presence of an infected focus which cannot be reached and treated surgically, a factor which may render a successful result quite out of the question. (2) The general condition of the patient : lowering of the general resisting power of the body by preceding long-continued illness seriously militating against recovery ; while very rapid, weak, irregular pulse, indicative of severe toxæmia of heart muscle and central nervous system, is of grave import.

Granting, then, that we have a patient suffering from acute septicæmia, with either no local focus of infection or with one which can be reached and immediately dealt with by the surgeon, with a high temperature, and rapid but reasonably strong and regular pulse, what procedure is to be followed ? Blood-cultures are to be taken, and an autogenous vaccine prepared with all speed. Not more than eighteen hours need be occupied over this. A skilled nurse is requisite, and the pulse-rate and rectal temperature must, if possible, be taken every two or three hours while the vaccine is being prepared. At the



top of an oscillation in the temperature a minimal dose of vaccine should be given—say 10,000,000 either of staphylococcus, streptococcus, *Bacillus typhosus*, or pneumococcus—and sleep secured with an adequate dose of hypnotic. The pulse-rate and temperature should be accurately taken every three hours without disturbing the patient. Within twelve to twenty-four hours the temperature and pulse-rate should be falling, and the pulse and respiration becoming stronger and more regular. Should one or more of these favourable symptoms not be discernible at the end of twenty-four hours, it is incumbent to administer a dose of double the size of the preceding, and wait another twenty-four hours. Should the temperature fall after the first injection, then it is well to wait till signs of a fresh rise appear, or until the pulse begins to accelerate or diminish in volume or regularity, when the initial dose should be repeated.

In the event of the increased dosage still being without effect at the end of twenty-four hours, no hesitation should be felt in again doubling it, proceeding to larger and yet larger doses.

With regard to indicial control, it may be admitted that, whenever it can be utilized, this should be done, for thus an additional guide to pulse, temperature, and general condition, is afforded.

In cases where pyrexia is slight, the value of indicial control becomes proportionately more marked; but, for my own part, I should attach much more importance to improved pulse and general condition than I should to raised opsonic index if these failed to show improvement. The great objections to the use of opsonic indices in acute septicæmia are twofold: (1) If they are to be of value, they must be very frequently



repeated, and the cost is heavy ; (2) the determination occupies considerable time, at the end of which the whole clinical picture may have changed. It is extremely unlikely that the index will remain stationary during the several hours occupied in its estimation ; the curve of immunity may have so changed that it may be inadvisable to make an injection at that time, and of this we have no power of cognizance. In brief, pulse, temperature, and general condition, are more reliable guides than opsonic indices in the vaccine treatment of acute septicæmia.

The reason why cases of general blood infection should respond well to vaccine treatment is as follows : The number of organisms present in the blood in such cases is relatively few ; but inasmuch as opsonin and probably other protective substances are not elaborated in the blood-stream, the presence of the organisms in the blood has no power of exciting the production of the protective substances which are lacking. The introduction of a dead culture into the subcutaneous tissues has, however, this effect, and the substances so elaborated are rapidly carried into the general circulation to make up the defect therein present.

The variety of staphylococcus usually present in septicæmia due to this organism is the *aureus*, but cases due to the *albus* are also on record. The author<sup>1</sup> has described the following case of systemic infection successfully treated by vaccines (fuller details will be found in the number of the *Lancet* referred to) : A boy, aged eleven, had eight weeks previously contracted scarlet fever. Apparent convalescence was followed by a fresh attack. At the end of a fortnight he was in a low muttering delirium, with a pulse of 128, somewhat irregular, but

<sup>1</sup> *Lancet*, September 11, 1909, p. 780.

full and fairly strong, and a temperature of 104° F. Both drums had perforated, and were discharging freely. Cultures, both from the ear and blood, showed *Staphylococcus aureus* and *Streptococcus conglomeratus*. Within twenty-four hours a vaccine of the former was prepared, and an injection of 8,000,000 given, along with 12,000,000 of a stock vaccine prepared from twelve strains of *Streptococcus conglomeratus*. The temperature and pulse began to improve almost immediately, as will be seen from the following table :

TABLE IX.

Date and Time of Injection.			Pulse.	Rectal Temperature.
				°F.
April 19,	8.30 p.m.	.. ..	128	104.8
April 20,	3 a.m.	.. ..	116	103.8
	7 a.m.	.. ..	120	103.6
	11 a.m.	.. ..	110	103.2
	3 p.m.	.. ..	108	103.0
	7 p.m.	.. ..	104	101.8
	11 p.m.	.. ..	110	102.8
April 21,	3 a.m.	.. ..	104	102.4
	7 a.m.	.. ..	116	100.0
	11 a.m.	.. ..	96	100.4

Consciousness was regained within twenty-four hours, sleep was good, and the following morning he was wanting to know what he could have to eat. The preceding injection was then repeated, and again three days later, the temperature oscillating daily between 99° and 101° F. Convalescence was uninterrupted, and fifteen days after the first injection the patient was up in a chair for two hours. The aural discharge rapidly lessened, but, to minimize the damage as much as possible, a few injections of 100,000,000 staphylococci and 25,000,000 streptococci

were given on the eleventh day of treatment, and at subsequent intervals of ten days. Ten weeks afterwards hearing was quite normal on both sides.

Although both the staphylococcus and the streptococcus were found in the blood, my impression was that the secondary invader, the *Staphylococcus aureus*, was the more important factor of the two. No indices were taken, immunization being carried out under the guidance of pulse, temperature, and clinical symptoms.

Whyte<sup>1</sup> details the result of a case in a man of fifty-six, with varicose ulcers of the leg and pyrexia, as high as 104° F., of three months' duration, with occasional rigors and night-sweats. A pure culture of staphylococcus was obtained from the blood, the index being 1·2. An injection of 250,000,000 was given, with the result that immediate improvement began. Next day the index was 0·7; subsequently it was 1·6, 1·8; and on the eighth day 2·6. Upon the tenth day the blood was sterile, and further treatment was unnecessary.

Turton (*loc. cit.*) records three cases. One of pyæmia originated in a septic osteomyelitis. There was a septic temperature, and multiple abscesses formed almost daily. Three injections of *Staphylococcus aureus* did not seem to have any influence, and the patient died nine days after the first injection.

The second case developed septicæmia a week after parturition. The temperature was 102° F. for five nights. Two injections were given at intervals of forty-eight hours. Twenty-four hours after the second injection the temperature dropped to normal, and recovery was uninterrupted.

The third case was subsequent to miscarriage. The pulse was 130, temperature 104° F., and the condition

<sup>1</sup> *Edinburgh Medical Journal*, December, 1907, p. 555.

desperate. Five injections were given at intervals of two to four days. She improved greatly, and eleven days after the first injection temperature was normal. Pulmonary embolism unfortunately supervened, and death resulted twenty days after the commencement of treatment.

Wright<sup>1</sup> also records a very interesting case. The patient suffered from mitral disease, and had had eight months of occasional pyrexia, with pulmonary infarction and cerebral embolism. A staphylococcus of somewhat aberrant characteristics was cultivated from the blood, and an 'autogenous' vaccine prepared. The experience with this was much more favourable than had been that with a stock vaccine previously employed. Six injections—three of 5,000,000 and three of 10,000,000—were given under the guidance of the opsonic index. The temperature soon became normal, and complete recovery ensued.

<sup>1</sup> *Lancet*, November 2, 1907, p. 1217.

## CHAPTER VI

### THE STREPTOCOCCUS

STREPTOCOCCI, like staphylococci, cause inflammation and suppuration in all parts of the body. They produce a hæmolysin, to which the anæmia present in some acute streptococcal infections is due, and to which human blood contains an antibody, and a toxin. Whether this latter be soluble—*i.e.*, extracellular—or not is uncertain; the probability is that it is an endotoxin. Against this toxin, whatever its true nature may be, the body is probably capable of elaborating an antitoxin.

Immunity against the bacteria themselves is secured by two agencies, bacteriolysis and phagocytosis, of which the latter is probably the more important (Meakins).<sup>1</sup> The opsonic index, accordingly, is a fair measure of the immunity, but as a rule temperature, pulse, and clinical symptoms, are sufficient guides in the conduct of immunization by means of a vaccine. Whether antitoxic serum or vaccine be the more valuable therapeutic agent cannot be considered to have yet been definitely established for each class of case. Where toxæmia is a very prominent symptom it is perhaps best to begin treatment with antitoxic serum, and proceed as rapidly as possible in the preparation of the autogenous vaccine, which may then be used in conjunction with the serum. That the term 'streptococcus' is but a generic name for

<sup>1</sup> *Journal of Experimental Medicine*, January, 1909, p. 100.

a large and heterogeneous group of organisms is now well recognized, and the failures so often experienced in the use of anti-streptococcal serum are very probably due to this fact, that even in the preparation of a polyvalent serum the particular variety causing the lesions under treatment had not been employed. No entirely satisfactory method of classifying the streptococci has yet been devised. Gordon<sup>1</sup> suggested that the reactions brought about in the following nine media should form the basis : Litmus milk, neutral red agar, and broth containing 2 per cent. of, respectively, saccharose, lactose, raffinose, inulin, salicin, coniferin, and mannite.

Andrewes and Horder have extended these by the addition of the following observations—viz. : growth on gelatine at 20° C., morphology in broth, and pathogenesis to the mouse. The results so obtained are set out in Table X.

TABLE X.

	S. Pyo- genes.	S. Sali- varius.	S. Angi- nosus.	S. Fæca- lis.	Pneumo- coccus.
Milk clot .. ..	..	+	+	+	±
Neutral red .. ..	..	+	±	±	..
Saccharose .. ..	+	+	+	+	+
Lactose .. ..	+	+	+	+	+
Raffinose .. ..	..	±	..	..	+
Inulin .. ..	..	..	..	..	±
Salicin .. ..	±	..	..	+	..
Coniferin .. ..	..	..	..	+	..
Mannite .. ..	..	..	..	+	..
Growth in gelatine at 20° C. .. ..	+	±	..	..	..
Morphology in broth ..	longus	brevis	longus	brevis	brevis
Pathogenesis for mouse	+	..	+	..	+

+ = Formation of clot in milk, and acid in other media.

<sup>1</sup> Local Government Board's Reports, 1903, 1904.

Horder<sup>1</sup> has investigated the variety of streptococcus present in eighteen cases of ulcerative endocarditis due to streptococci. The majority of these proved to belong to the *salivarius*, and *fæcalis* types, indicating that infection has occurred either via the tonsil or intestine. The cases in which the *Streptococcus pyogenes longus* occurs are relatively few, and belong to the fulminant type, being usually rapidly fatal. Why the other cases run such a prolonged course and exhibit so few signs of auto-intoxication, although bacteria may be present in the blood in large numbers, is evident from consideration of this fact, that the infecting organisms are the comparatively non-virulent streptococci of the salivary and intestinal types.

Litman and Celler found in 277 cases of acute otitis media that the *Streptococcus pyogenes longus* was present in 81 per cent., the *Streptococcus salivarius* (or *mucosus*) in 10 per cent., and the pneumococcus in 9 per cent. The percentage figures for the pneumococcus are, however, probably much too low.

Wilson<sup>2</sup> isolated the streptococci from five cases of streptococcal meningitis; two of these strains were not fully studied, owing to death of the cultures. Of these one was probably pneumococcus; the other was not *fæcalis*, but possibly *pyogenes longus*; the other three appeared to belong to the *fæcalis* group.

The variety usually present in ulcers and bone abscesses appears to be the *pyogenes longus*. The advisability of employing the autogenous vaccine prepared from the patient's own individual strain in every case of streptococcal infection is thus evident. Should this not be

<sup>1</sup> *Practitioner*, May, 1907, p. 715.

<sup>2</sup> *Lancet*, December 28, 1907, p. 1816.



possible, then a highly polyvalent vaccine prepared from at least six different strains should be used. At the same time it may be mentioned that Tunnicliff<sup>1</sup> has found that in acute articular rheumatism the indices for the *Streptococcus rheumaticus* and *pyogenes longus* ran an identical course, but that, on the other hand, the index towards pneumococcus and a strain of *Streptococcus mucosus* isolated from a normal throat remained within the normal limits.

#### STREPTOCOCCAL SEPTICÆMIA AND PYÆMIA.

Several cases have now been recorded in which vaccine therapy by means of an autogenous vaccine has been resorted to in this variety of systemic infection. The first case was described by Sir James Barr before the Liverpool Medical Institute on May 3, 1906, the treatment having been conducted by Captain Douglas. It was a very severe case, rigors occurring every twenty-four hours or oftener, when the temperature mounted to 104° or 105° F., and was followed by profuse sweating. There was an attack of pleurisy with pleuro-pericardial friction, but without marked effusion. All the various brands of anti-streptococcal serum had been tried without much benefit.

Douglas isolated the streptococcus from the patient's blood, and a vaccine was made. The index was estimated twice daily, and injections given of 5,000,000 to 12,000,000 cocci at each fall of the index.

The patient made an excellent recovery, and, except for the effects of phlebitis in the left lower limb, was soon quite well.

<sup>1</sup> *Journal of Infectious Diseases*, June 12, 1909, p. 346.

Sutcliffe and Bayly<sup>1</sup> have described a case of streptococcal septicæmia in a boy of fourteen, who had been operated on for discharging tubercular glands, which was successfully treated with a streptococcal vaccine. Pus was found along the track of the right deep femoral vein, and there was threatened formation in a similar situation in the left thigh. The cocci were isolated from the blood, and the index found to be 0·66. An injection of 10,000,000 organisms raised the index to 1·15 by the following day. In the course of forty-five days ten injections, varying in amount between 10,000,000 and 50,000,000 organisms, were given. Reduction of temperature, elevation of the index, and general improvement in the patient's condition, ensued after each injection, and complete recovery was the ultimate result. Upon two occasions, when manipulation of the limb was performed, considerable depression of the index, due to auto-inoculation, was observed the next day.

Turton (*loc. cit.*) has had experience of three cases.

The first had received a scratch from a dirty wire, there was œdema and redness of the hand and arm, and great prostration with a temperature of 102° F. Injections were given upon the third, fifth, eighth, and twelfth days after infection, and an excellent recovery ensued.

Case number two, also of septicæmia, had a favourable issue after four injections spread over seven days.

The third was one of puerperal septicæmia, which seemed hopeless from the first. Two injections on the ninth and eleventh days after parturition seemed to produce no good result.

Crowe and Wynn<sup>2</sup> give the following interesting account of a case of puerperal septicæmia due to mixed infection

<sup>1</sup> *Lancet*, August 10, 1907, p. 367.

<sup>2</sup> *British Medical Journal*, August 8, 1908, p. 303.

by streptococcus and *Bacillus coli communis*, and therefore of bad prognosis. Upon the ninth day after labour the temperature rose to 99·6° F., and clots came away; on the fifteenth, sixteenth, and seventeenth days the temperature rose to 100·4° F., and upon the eighteenth to 101° F., when *Streptococcus pyogenes longus* and *Bacillus coli communis* were isolated. Upon the eighteenth, twentieth, twenty-first, twenty-second, and twenty-fifth days 10 c.c. of anti-streptococcal serum were given without any good result. On the twentieth day the index to *Streptococcus pyogenes* = 0·65. Upon the twenty-fifth day 70,000,000 of *Bacillus coli communis* and 10,000,000 of streptococcus of autogenous vaccines were given. In the evening the temperature rose to 102° F., but on the third morning it fell to normal, the indices being then to *Bacillus coli communis* 1·58, to streptococcus 1·72. The temperature remained normal for four days, then rose to 100° F. This was recognized as being due to two carious teeth, with abscesses at their roots. These were extracted, and recovery proceeded uninterruptedly.

Wright<sup>1</sup> relates his experiences in six cases of streptococcal septicæmia (one of these being the case of Barr and Douglas, already described). Of these, two cases were cured, having made very satisfactory immunizing responses; another made a very good response, but died from cardiac complications four days after defervescence. Three cases died without making any immunizing response to the inoculations, despite the use of doses varying greatly in magnitude.

Reference may also be made to the author's case of double septicæmia produced by the *Staphylococcus aureus* and *Streptococcus conglomeratus*, described on p. 97.

<sup>1</sup> *Lancet*, August 24, 1907.

Results such as those recorded above, in a complaint of such grave prognosis as streptococcal septicæmia, in which the mortality appears to be about 95 per cent., can only be described as highly satisfactory. Horder, however, takes a very gloomy view of the prognosis in cases of infective endocarditis. Ten under his care all died ; but the adoption of his own suggestions of pushing the dosage and simultaneously employing antitoxic serum would probably yield much better results.

In cases such as have been described under this heading adequate control of the course of immunization by means of opsonic index determinations is hardly practicable, inasmuch as the index varies with such rapidity that long ere the estimation can be completed it will probably have considerably altered.

The quality and rate of the pulse, the temperature, and general condition, are probably sufficient guides as to when the initial dose of 5,000,000 to 10,000,000 should be repeated or increased. Slowing of the pulse, better volume and increased regularity, lowering and steadying of the temperature, mental quietening and inclination for sleep, are the signs that satisfactory progress in immunization is being secured.

In erysipelas, empyema (of which 55 per cent. in adults and 15 per cent. in children Netter has shown to be due to streptococci), secondary joint infections, impetigo, dacryocystitis, ulcers, and infected wounds and endometritis, the *Streptococcus pyogenes longus* is, as a rule, the variety present. Initial doses of 10,000,000 to 20,000,000 may be employed, and a completely satisfactory result may be anticipated.

## ERYSIPELAS.

Schorer<sup>1</sup> studied the index in erysipelas, and the effects of therapeutic inoculation by vaccines, and came to the following conclusions :

1. The onset of an attack causes rise of index, which attains a maximum about the third day, and then gradually falls.

2. No constant change in index occurs at the time of desquamation, and only half the patients discharged as cured have a higher index on discharge than they had on admission.

3. Injections of 25,000,000 cause a rise of index after twenty-four hours without any preceding negative phase. Next day there is a slight fall, but a raised index is maintained for about seven days. With a dose of 50,000,000 the rise is delayed till the second day.

4. Inoculation does not prevent migration or recurrence, but seems to shorten the duration of an attack by about three days.

5. The index is so variable that it is of no use as an indication of the severity of the disease, nor is it of value in prognosis.

6. No relation is to be observed between elevation of the index and improvement in the patient's clinical condition.

Other observers have obtained much more favourable results, and have come to quite different conclusions. Thus, Ross and Johnson<sup>2</sup> treated fifty cases with a vaccine of *Streptococcus erysipelatis*. In severe cases the initial dose was 10,000,000, in milder ones 20,000,000. The most striking change which they observed was rapid

<sup>1</sup> *American Journal of Medical Science*, November, 1907, p. 728.

<sup>2</sup> *Journal of the American Medical Association*, March 6, 1909.

subsidence of the severe toxæmic symptoms. There was generally a spread of the local process for twenty-four to forty-eight hours, but it was brownish rather than red, while the swelling and tenderness were less, and the margin soon began to fade.

Butler Harris<sup>1</sup> records the following: From a case of severe facial erysipelas an autogenous vaccine was prepared and administered upon the sixth day, when the temperature was 105·4° F., the pulse 140, respiration 45, and the patient in imminent peril. Recovery resulted by crisis, the temperature dropping to 98·8° F. fourteen hours after inoculation.

Wynn<sup>2</sup> has described the following two cases. The first, a medical man, infected his finger at a post-mortem upon a case of ulcerative endocarditis. The infection quickly spread from the finger to the axillary glands and subcutaneous tissue of the arm. Severe erysipelas extended all over the trunk and down the thighs, and the patient became dangerously ill. From the *Streptococcus longus* isolated a vaccine was prepared. The opsonic index rose considerably after the first inoculation, and other inoculations were given, when daily estimations showed the index to be falling. Good response followed each administration. Prior to the first injection, which was given upon the sixteenth day, the temperature had showed daily rises to 102° and 103° F., and on the thirteenth day to 105° F. Subsequent inoculations were given on the eighteenth, twenty-first, and twenty-seventh days. Upon the twenty-fourth day the temperature fell to normal, and the patient made a good recovery.

The second case was one of severe facial erysipelas.

<sup>1</sup> *Practitioner*, May, 1908, p. 647.

<sup>2</sup> *Birmingham Medical Review*, June, 1908.



The temperature showed daily rises to 104° F., and once to 106° F., with remissions to 100° and 101° F. Inoculations of 10,000,000 were given on the third, fifth, tenth, thirteenth, and fourteenth days of the disease. The temperature dropped by crisis on the day following the last inoculation.

#### RHEUMATISM AND CHOREA.

Poynton and Paine have described a form of streptococcus as being the probable causative agent in acute rheumatism. It exhibits no definite distinctive reactions. It is a very small diplococcus, growing best in a mixture of equal parts of milk and broth. It has strong acid-forming tendencies, fermenting glucose, lævulose, galactose, maltose, arabinose, dextrin, saccharose, lactose, salicin, and mannite, but not inulin, dulcitol, or sorbitol. It turns milk acid, but does not as a rule form a clot. It forms acid in bile salt lactose broth, precipitating the litmus and bile salts. When grown in broth, it forms considerable quantities of formic acid (Ainley Walker); if the broth be then filtered through a porous porcelain candle, the *Streptococcus rheumaticus* will fail to grow in the filtrate, while other forms of streptococcus will grow well. The identity of this organism is still, however, disputed.

Fordyce<sup>1</sup> observed the opsonic index towards this organism in one case of acute rheumatism with pyrexia, painful and swollen joints, acute pericarditis, and albuminuria. Two days after admission the index = 0.59. During this day the fever and the physical signs of pericarditis subsided. Next day the patient was markedly better and the index = 1.1; for the heated serum it was 0.45 as compared with 0.1 for a heated normal serum.

<sup>1</sup> 'International Clinics' (eighteenth series), vol. i., p. 40.



The patient steadily improved, and six days after admission the index = 1·3.

In ten cases of chorea he found the index, as a rule, low upon admission, rising later as the general health improved. The lowest index found = 0·6, the highest 1·8. Upon several occasions the index was normal, and all such indices occurred when improvement in the clinical symptoms was taking place, and upon more than one occasion was an intermediate step from a low to a high index.

Tunncliffe<sup>1</sup> finds that in acute articular rheumatism the index towards the *Streptococcus rheumaticus* follows a definite course. With involvement of new joints and rise in temperature the index falls; with improvement in the joints and symptoms it rises above normal. The index towards the *Streptococcus pyogenes*, but not that towards other varieties of streptococcus, follows an identical course. In cases which end fatally a preagonal rise of the index may be exhibited.

#### STREPTOCOCCI IN SCARLET FEVER.

The view has steadily grown in favour of late that streptococci are intimately connected with the complications of scarlet fever, if not with the pathogenesis of the fever itself. Thus, in over 70 per cent. of cases with albuminuria, streptococci are copiously voided in the urine, and in about 15 per cent. of cases without albuminuria. Bearing this in mind, Banks<sup>2</sup> has studied the variations in the opsonic index of the blood to streptococci as the disease progressed. He found that in cases running a fairly normal course the opsonic power towards streptococci varies in a pretty definite and constant way.

<sup>1</sup> *Journal of Infectious Diseases*, June 12, 1909, p. 346.

<sup>2</sup> *Journal of Pathology and Bacteriology*, October, 1907, p. 113.

It is decreased during the early febrile period, and rises to normal or above normal during the defervescence and general decline of symptoms. It falls during the second and third weeks, and even in uncomplicated cases the index may be comparatively low. There is an increase to normal or over during the fourth and fifth weeks. In fatal cases with severe angina the opsonic power is markedly subnormal. Complications alter the usual curve, causing both absolute and relative differences. Thus, the opsonic power is decreased at the onset and during the earlier period of albuminuria and secondary adenitis; as convalescence is established the index rises. The opsonic values do not furnish many data for prognosis, but, in general, a persistent low index during nephritis or other serious complication is an unfavourable sign.

The author therefore thought it worth an effort to determine whether the course of scarlet fever could be favourably influenced by the administration of a vaccine prepared from the streptococci isolated from the throats of scarlet fever patients. From the throats of each of twelve such cases the *Streptococcus* 'conglomeratus,' both of the small and large types, was recovered, and with great difficulty a mixed vaccine was prepared. The effect of this was then tested, in conjunction with Dr. Goodall, in six cases of uncomplicated scarlet fever, but apparently without producing the slightest good effect. Doses varying from 10,000,000 to 50,000,000 were employed. This result, although discouraging, is of course not conclusive, and it is quite possible that good may be effected in cases complicated by angina, adenitis, and nephritis, as there is little doubt of the streptococcal nature of these complications.

## CHAPTER VII

### THE PNEUMOCOCCUS

AMONG the pathological conditions amenable to vaccine treatment for which the pneumococcus is responsible may be mentioned Pneumonia, Pleurisy, Pericarditis, Empyema (both pulmonary and of the accessory air sinuses), Peritonitis, Otitis, Meningitis, Conjunctivitis, Ulcus Serpens Corneæ, Arthritis, Periostitis, Nephritis and Perinephritis, Metritis and Pyosalpinx, Abscesses, Septicæmia, and Pyæmia. Its connection with ulcerative pharyngitis has been described by Semon<sup>1</sup> and by Elliott,<sup>2</sup> while its importance in one of the severer forms of catarrh of the respiratory tract, in bronchitis and asthma, will be explained more fully in Chapter X.

Whether the pneumococcus forms exotoxin or not is not yet settled, but the probability is that it may to a limited extent; a hæmolysin and a leucocidin are produced by some varieties, and not by others. Its pathogenic effects are therefore mainly due to endotoxin. To these toxins antibodies may be formed as the result of artificial immunization, but are apparently not present in cases of infection in human beings. Agglutinins are usually then found to a limited extent, but appear to be of little significance. Recovery in pneumococcal infections seems to be brought about largely by the action

<sup>1</sup> *Medical Magazine*, December, 1908.

<sup>2</sup> *British Medical Journal*, June 26, 1909, p. 1528

of opsonins, more especially of thermolabile opsonin, although the amount of thermostable opsonin is also increased. The opsonic index is therefore a more than usually accurate guide to the progress of immunization ; but whether its value in febrile cases is greater than that of pulse, temperature, and general condition, has not yet been determined. Personally, I should always be more than glad of its guidance, but feel that in the great majority of cases it may be dispensed with. Afebrile cases are usually localized, and in these regard must be paid to clinical symptoms, and careful microscopical examinations of the secretions must be made.

A vaccine of the dead cocci is but slightly toxic, and produces its effect through increasing the formation of opsonin, especially of the thermostable variety. The various sera upon the market (Pane's, Römer's, Klemperer's) possibly act in a similar manner rather than by means of any antibodies which they may contain.

### PNEUMONIA.

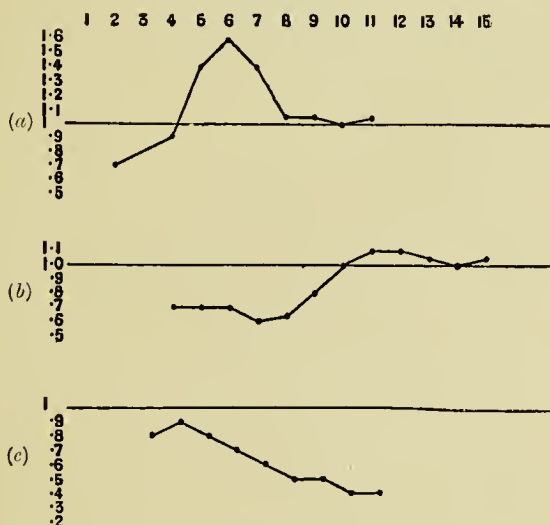
MacDonald<sup>1</sup> and Eyre<sup>2</sup> have made a careful study of the opsonic index during the course of an attack of pneumonia. While the temperature is rising and during the fastigium the index is below normal, whereas at the onset of the crisis there is a sudden rise, especially in the amount of the thermolabile opsonin. When recovery takes place by lysis, the rise in index is gradual, while in cases which progress to a fatal issue there is a steady fall. Failure, therefore, of the index to rise at the crisis is of

<sup>1</sup> Pathological Society, London, January 17, 1905 ; Pathological Studies, University of Aberdeen.

<sup>2</sup> *Lancet*, February 22, 1908.

grave prognostic import. These facts are diagrammatically expressed in Chart No. 12, while the relationship between number of leucocytes, opsonic index, and temperature in a case of pneumonia running its usual course is well shown in the following chart, No. 13, prepared by Dr. J. W. Eyre.

CHART XII.

*Days of disease*

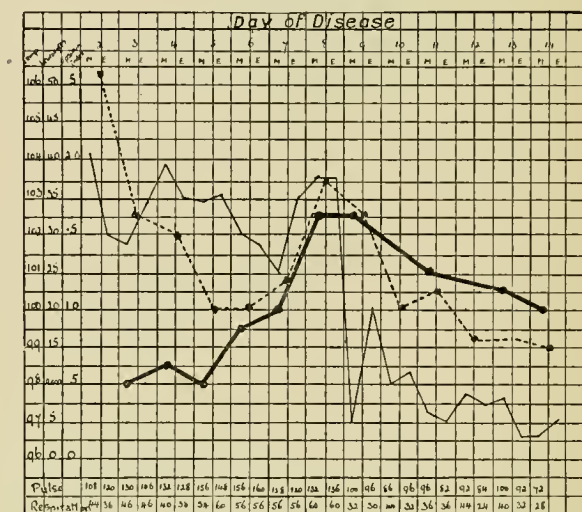
TYPES OF REACTION OF THE OPSONIC INDEX IN PNEUMOCOCCIC INFECTION. (After Eyre.)

(a) Immediate, as seen in mild diseases ; (b) delayed ; and (c) progressive decline, as seen in severe and fatal infections.

Reference has already been made to the observation of Rosenow, that the leucocytes of a case of pneumonia have greater phagocytic powers than normal (see p. 30) ; they are also less easily killed by heat. Thus, the defensive mechanism appears to be compounded of the three factors, increased opsonin, increased number of the leucocytes,

and increased phagocytic power of these latter ; and of the three, the most important appears to be the opsonin. Logically, therefore, in all cases of pneumonia the physician's endeavour should be to aid all three factors in recovery. In practice, the leucocytes are as a rule adequately increased in numbers ; in cases where there is

CHART XIII.



RELATION BETWEEN LEUCOCYTES, OPSONIC INDEX, AND TEMPERATURE, IN A CASE OF PNEUMONIA. (Eyre.)

Dotted line = number of leucocytes per cubic millimetre ; thick line = opsonic index ; thin line = temperature.

no leucocytosis, or even a leucopenia, the prognosis is much less favourable, as is well known. Unfortunately, there is little reason to anticipate benefit from such artificial provocatives of leucocytosis as injections of cinna- mate of soda ; nevertheless, the attempt may be made.

Again, a method of increasing the phagocytic power

of the leucocytes has not yet been discovered, unless injections of fresh horse serum have this power. We are therefore limited, in our endeavours to find a truly scientific method of treating cases of pneumonia, to increasing the amount of opsonin ; and this, as has been already shown, can be effected by injections of a vaccine, best of the patient's own bacteria ; the benefits thereby secured are so immediate and so great that the author has little diffidence in asserting that the space of a few years will see all cases of pneumonia treated by this method. Within a few hours—twelve to eighteen—of an injection of 10,000,000 to 25,000,000 of an autogenous vaccine, the temperature begins to fall, the pulse is slower, stronger, and more regular, the physical comfort and well-being of the patient is increased. The temperature should be taken four-hourly, without disturbing the patient more than possible, and the rectal method is to be advocated. Any abrupt rise of over  $1.5^{\circ}$  C. should be the signal for renewing the injection.

The most important points in prognosis are—(1) the pulse, (2) the general condition.

A weak, irregular, very rapid pulse, enfeebled constitution, low muttering delirium, dry furred tongue, and sordes about the lips, are of course unfavourable signs ; yet so marked has been the improvement, even after one injection, in two cases of this type, that no case is to be looked upon as hopeless.

Butler Harris,<sup>1</sup> from a study of eleven cases of pneumococcal lung infections treated with vaccine, concludes—

1. That successful inoculation for pneumonia is possible.

2. That inoculation does no harm.

<sup>1</sup> *British Medical Journal*, June 26, 1909, p. 1530.



3. That a vaccine from one or a number of *virulent* strains should be used.

4. That it should be introduced as early as possible.

5. That indical control is unnecessary.

6. That temperature and physical signs are sufficient guide.

The advantages, then, to be claimed for routine treatment by vaccines in all cases of pneumonia are : Precipitation of the crisis—diminution, therefore, of the risks of heart failure and other toxæmic symptoms ; increased comfort of the patient, and diminished liability to such sequelæ as empyema ; curtailment of the whole course of the disease to a fortnight, or even less.

In chronic or unresolved pneumonia the exhibition of a vaccine is the treatment *par excellence*. An initial dose of 25,000,000 may be increased subsequently to 50,000,000, 100,000,000, 250,000,000, and even 500,000,000, the control for dosage and intervals between the injections being furnished by—(1) auscultatory signs ; (2) careful microscopy of the sputum for bacteria and varieties of cells present ; (3) general condition of the patient.

The presence of an empyema or septic pericarditis will, of course, necessitate surgical interference, and render the prognosis less favourable.

Numerous cases of vaccine treatment of chronic pneumonia have now been recorded, of which mention may be made of the following :

*Case of Delayed Resolution after Pneumonia.*<sup>1</sup>—Four months prior to commencing vaccine treatment pneumonia had supervened upon septic tonsillitis. The crisis occurred upon the thirteenth day, but little resolution had resulted. Numerous large and small râles were to be heard

<sup>1</sup> Allen, *Lancet*, September 11, 1909, p. 781.

all over the left chest, and there was copious thick, yellow expectoration. Pneumococcus and *Micrococcus catarrhalis* were isolated, and a vaccine of each prepared. The following injections were given :

On October 2, 1908, 50,000,000 of each : no pulse or temperature reaction followed.

On October 17, 50,000,000 pneumococci, 125,000,000 *M. catarrhalis* : no reaction.

On October 26, 100,000,000 pneumococci, 125,000,000 *M. catarrhalis*.

Considerable improvement had already resulted ; only about a teaspoonful of sputum was voided daily, and this contained relatively few pneumococci, while the chest was clearing rapidly.

On November 12 and 24, 250,000,000 of both pneumococci and *M. catarrhalis*.

On the latter date there were only a few râles at the base.

On December 10, 250,000,000 of each.

Only one lump of sputum, containing a very few pneumococci, had been expectorated during the few days preceding, and nothing was to be heard except a fine mucous click after a deep inspiration. The patient has remained well, but still has an occasional dose of her pneumococcal vaccine, as this, she finds, checks a tendency to recurrent pharyngitis and laryngitis.

Coleman recorded before the Royal Academy of Medicine, Ireland, on March 2, 1906, a case treated by inoculation of pneumococcal vaccine, with very satisfactory results.

On the thirty-eighth day of attack the pneumococcic index was 0.6 ; 46,500,000 cocci were therefore given. There was no disturbance, local or general. Next day the

index was 0·69, and the physical signs were those of pneumonia of five or six days' standing.

6 days after injection the index=1·17, and the patient was much better.

10 days after injection the index=0·89, and 46,500,000 were again given.

3 days after second injection the index=1·13.

Eleven days after the second injection the patient was in excellent health, and for six weeks subsequently the index was observed to be slightly over normal.

Briscoe and Williams<sup>1</sup> subjected two such cases, uncomplicated by empyema, which were not improving under ordinary treatment, to vaccine therapy. Cultures were made from the patients' own organisms, and the guidance of the opsonic index was utilized.

Case 1 was in a child aged one and a half years. A month after admission a consolidation at the right base was still unresolved. The index being 0·9, an injection of 20,000,000 cocci was given. A slight rise of temperature resulted, and the child was not so well for eighteen hours. He then began to improve in weight and general condition.

2 days after the first injection the index=1·2.

4 days after the first injection there were only a few crepitations and slight bronchial breathing.

5 days after the first injection the index=1·3.

6 days after the first injection there were crepitations, but no bronchial breathing

9 days after the first injection the index=1·2.

11 days after the first injection 10,000,000 cocci were given without any ill effect, and next day index=1·4

2 days after the second injection crepitations were audible only at lower and posterior aspect of the lobe.

5 days after the second injection 10,000,000 cocci were given, and next day the chest was clear.

<sup>1</sup> *Practitioner*, May, 1908, p. 675.

Case 2 was one of right basal consolidation with a history of one week in a man of forty-four. Although the temperature soon fell to normal, the local signs failed to clear up. Sixty million organisms were therefore injected on about the twenty-second day, and again thirteen days later. After the first injection the moist sounds cleared up entirely in the next three days, and the sputum diminished two days after the second injection. There was no bronchial breathing, but the breath sounds were a little harsh. His general and mental condition, previously bad, improved rapidly.

They also produced decided improvement in two other cases of a chronic nature in adults, the history in each dating back ten months. Doses of 50,000,000 and 100,000,000 were employed.

The authors' conclusions are that 'in these more or less acute cases the improvement in general condition is quite a marked feature, and it appears to be an important factor in the question of continuing the treatment. The injection produces a stimulating effect, and the patients always seem to be more cheerful afterwards. An increase of weight occurs rapidly in the case of children.'

#### EMPHYEMA.

With regard to this complication of pneumonia, it must especially be borne in mind that mixed infection by the streptococcus, staphylococcus, *Bacillus coli communis*, *B. pyocyaneus*, and other less common organisms, is very frequent—nay, sometimes the secondary infection has supplanted the primary one altogether. The haphazard administration of a pneumococcal vaccine in cases of empyema cannot, therefore, be too strongly condemned. Most careful examination, microscopically and

culturally, of the sputum and pus should be made, to determine the precise nature of the infection, and to indicate whether one or more vaccines are required. Here, as always, the necessity for free drainage can hardly be too strongly emphasized; but should operation not be possible, no hesitation need be felt in proceeding to vaccine treatment. The initial dose for children is 5,000,000 to 10,000,000; for adults, 25,000,000 to 50,000,000. When the general condition is bad, the smaller alternative dose should be employed.

For some good instances of the complicated bacteriology of many cases of empyema, and for the results of treatment in certain instances, the reader is referred to a paper by Hale White and Eyre.<sup>1</sup>

In dealing with these cases it is to be remembered that improvement may be slow, and prolonged treatment be necessary. In cases of mixed infection, the bacteriological picture may change from time to time, fresh invaders making their appearance, and for these an additional vaccine may be necessary. Extreme care must therefore be taken to diminish this risk as much as possible by the use of stringent antiseptic measures whenever there is an opening from the empyema.

#### SEPTICÆMIA, ETC.

Realization of the frequency with which the pneumococcus is responsible for acute catarrhs of the respiratory tract, such as acute colds, bronchitis, pharyngitis (ulcerative and non-ulcerative), renders easy of understanding the involvement of the middle ear or the occurrence of septicæmia without a precedent pneumonia. An excel-

<sup>1</sup> 'Vaccines in General Medicine,' Proceedings of the Royal Society of Medicine, June, 1909, Therapeutical Section, p. 150.

lent example of the latter is to be found in a paper by the author in the *Lancet* (September 11, 1909, p. 780). Not only was there a general septicæmia, but multiple abscesses in the muscles and joints were also present. Despite the gloomy nature of the prognosis, the patient made a complete recovery, doses of 50,000,000 to 250,000,000 of an autogenous vaccine being employed.

Warren Low<sup>1</sup> describes a most interesting case of pneumococcal arthritis and cystitis. There was here a history of so-called 'influenza'—in reality, a pneumococcal cold. Tendon-sheaths and various joints were affected. There were numerous small ulcers in the bladder, and recurrent swelling of the prepuce. Injections of 5,000,000 to 20,000,000 of an autogenous pneumococcal vaccine were given at intervals of about seven days. The patient was convalescent in about six weeks. Recovery was complete.

To four cases of pneumococcal infection of special interest brief reference may be made. The first<sup>2</sup> was a case of primary diffuse peritonitis in a child of eight, treated by operation and vaccine at intervals of five to six days in doses of 10,000,000 to 25,000,000. The opinion of the surgeon was that the vaccine contributed largely to the successful issue.

The second was a case of pneumococcal peritonitis secondary to an infected Fallopian tube in a girl of fourteen, recorded by Towers.<sup>3</sup>

The third,<sup>4</sup> a case of appendical abscess due to mixed

<sup>1</sup> Proceedings of the Royal Society of Medicine, June, 1909, Clinical Section, p. 231.

<sup>2</sup> Robinson, *British Medical Journal*, March 13, 1909, p. 651.

<sup>3</sup> *Practitioner*, September, 1908.

<sup>4</sup> Harrison and Turton, *British Medical Journal*, May 1, 1909, p. 1054.



infection by pneumococcus and *Bacillus coli*. A study of the opsonic chart leads to the opinion that the pneumococcal was the more important infection. After operation the patient remained in a very critical state, with symptoms of severe toxæmia. An injection of 25,000,000 of an autogenous pneumococcal vaccine and of 50,000,000 of one of the *B. coli* caused a fall of temperature from 103° F. to 99.5° F., and a rise within two days of the pneumococcal index from 0.81 to 1.28.

The fourth<sup>1</sup> was a case in which the evidence of acute infective endocarditis was strong. An injection of 50,000,000 of an autogenous vaccine was given when the temperature was 102.2° F., the pulse 156, and the patient very restless and irritable. In half an hour the patient was asleep, and only woke once in six hours. He then said he felt stronger and in less pain. In four days the temperature was normal and the pulse 100. The following day the injection of 50,000,000 was repeated, while four and seven days later this was increased to 100,000,000. The patient made a complete recovery.

Rosenow<sup>2</sup> finds that in cases of infective endocarditis and septicæmia the bacteria are of low virulence,<sup>3</sup> but that they appear to immunize themselves against the phagocytes of their host. Hence the difficulty of a natural cure. Vaccine treatment he has found disappointing, although late in the course of this disease temporary improvement may result.

For *ulcus serpens corneæ* and pneumococcal conjunctivitis, see Chapter XIII.

For pneumococcal catarrh of the respiratory tract and its accessory air sinuses, see Chapter X.

<sup>1</sup> Conder and Collins, *Practitioner*, August, 1909, p. 203.

<sup>2</sup> *Journal of Infectious Diseases*, April 1, 1909, p. 245.

<sup>3</sup> Compare Horder on the Streptococci, p. 103.



## CHAPTER VIII

### THE GONOCOCCUS

THE chief conditions set up by this organism are Urethritis, Periurethritis, Prostatitis, Vesiculitis, Cystitis, Epididymitis and Orchitis, Endometritis, Salpingitis, Peritonitis, Conjunctivitis, Endocarditis, Arthritis, and even Pleurisy and Septicæmia. In these connections a very wide field of utility is afforded, both in diagnosis and treatment.

The pathogenic effects produced by this organism are almost entirely due to an endotoxin of extreme stability, the formation of exotoxin and hæmolysin being both doubtful.

The defensive mechanism of the body against the gonococcus is little understood. Torrey has succeeded in producing both bacteriolysin and agglutinin by the artificial immunization of animals; but these do not appear to be formed in the human subject—at least to any appreciable extent. Loss of virulence of the organism, acquired local immunity, and exhaustion of a necessary nutrient material, have been suggested as playing a part, but of these no proof has been adduced. The fact that much the greater proportion of the gonococci in the urethral secretion are always intracellular, both in cases which rapidly recover and in those which subsequently become chronic, would appear to indicate that phagocytosis and opsonin formation are not very important factors in the production of immunity. One fact in this connec-

tion appears, however, to have escaped notice ; it is this : Film preparations from the urethral secretion of two early cases of gonorrhœa may show equally good phagocytosis of the organisms, but cultures of the pus from one of these cases may grow readily, the other poorly or not at all, although intracellular digestion of the cocci may not be obvious. I have endeavoured to make out the significance of this phenomenon, and believe that, in those early cases where cultures are difficult to obtain, cure is much more rapid than in the greater proportion of those of the former class, where good growth occurs. If this be so, then, as with the pneumococcus, variation in the leucocyte is a most important factor. Be this as it may, it yet remains that in a vaccine an important aid is found for the treatment of gonococcal infections, probably through increasing the elaboration of opsonin.

#### THE OPSONIC INDEX IN GONOCOCCAL INFECTIONS, AND ITS UTILITY IN DIAGNOSIS AND TREATMENT.

In acute gonorrhœal infections of the urethra, the index as a rule first falls for a few days to 0·6 or 0·7 ; it may then either rise steadily to 1·3 or 1·6, such cases usually doing well under routine treatment, or it may continue subnormal, when they usually pass on into a chronic intractable gleet.

In chronic cases the index is usually low, even 0·3 ; it is, however, sometimes normal or above normal, but in these cases cocci, as a rule, are to be found copiously in the secretions from suppurating Littre's glands, lacunæ, or sinuses, which may continue thus to discharge at intervals for many years.

In acute gonorrhœal conjunctivitis in adults the index may be as high as 2, or even 2·5.

Every genito-urinary surgeon and obstetrician is familiar with the great difficulty of deciding whether an old gonorrhœal infection has disappeared, or of arriving at a diagnosis in cases where a history of an acute attack is not obtainable. In the male it is no very uncommon thing for a discharge to persist even for ten years after an attack of acute gonorrhœa. Stained films do not reveal the presence of any gonococci, but only of streptococci, staphylococci, the bacillus of Friedländer, the *Micrococcus catarrhalis*, *Bacillus coli*, a small Gram-negative organism very closely resembling the *B. influenzae*, and other + and - Gram organisms. The difficulty of advising as to the safety or otherwise of marriage in these cases is considerable. In deciding whether there are any latent gonococci encapsuled in the numerous urethral crypts and diverticula I have found the opsonic index of the utmost assistance. Brief references to a few cases will illustrate this.

Case 1 had a chronic discharge for ten years. No gonococci could be found in films, but the bacillus of Friedländer was present in vast numbers in a state of purity.

The gonococcal index was 1.1, that towards the bacillus of Friedländer 0.6. Non-gonococcal infection was diagnosed, and treatment by means of a vaccine prepared from the pneumobacillus carried out with complete success.

Case 2 was one of twelve years' standing, which had proved obdurate to every form of treatment. No gonococci could be detected in smears or cultures, and the index was 1.2. The patient desired a course of injections with gonococcal vaccine, and five were accordingly given, but, as anticipated, without influencing the discharge.

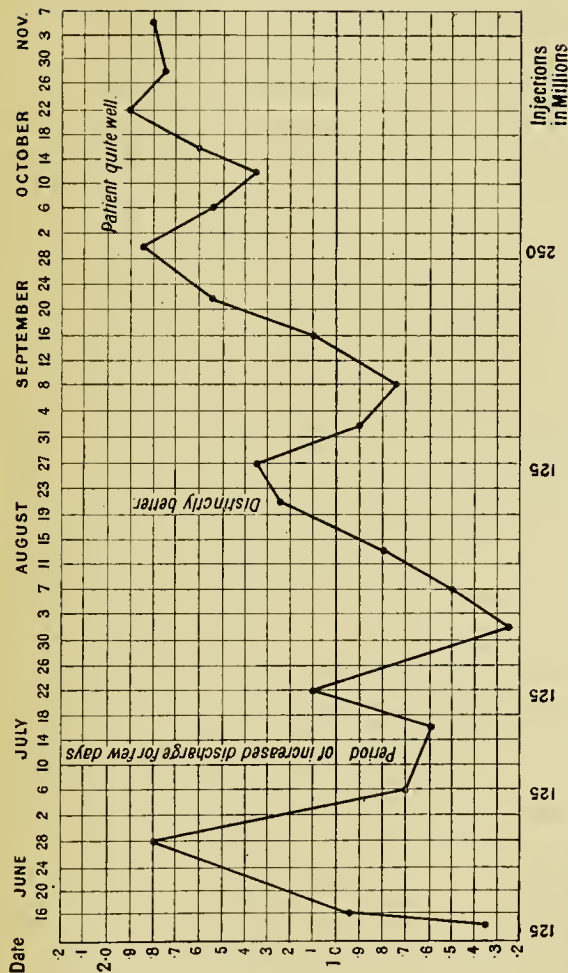
A combined vaccine was then prepared from the urethral organisms present ; two injections sufficed to reduce these greatly in numbers and variety, and a fresh vaccine was then made. Two injections with this resulted in great diminution of the discharge, which contained only epithelial and lymphoid cells and few organisms, which were contained within the epithelial cells, and proved very difficult to cultivate. Subsequent irrigation for a few days with weak solutions of perchloride of mercury completely cured the discharge.

In these two cases, then, the gonococcal index was normal, and the diagnosis of non-gonococcal infection was amply confirmed by the results of treatment.

Cases 3 and 4 were each of ten years' standing, and had undergone the most expert surgical treatment, both in England and upon the Continent, but without avail ; exacerbations appeared from time to time without any obvious cause. In Case 3 no gonococci were to be found, in Case 4 only at intervals. The indices were found to be 0.4 and 0.5 respectively ; gonococcal infection was therefore diagnosed, and vaccine treatment advised, with complete success, two injections sufficing in the instance of Case 4.

Case 5 was sent me by Mr. Wyndham Powell, and is peculiarly interesting. The attack was a first one. Intercourse, which had taken place seven days previously, was followed by discharge three days later. The patient at once saw Professor Janet, who diagnosed a simple non-gonococcal urethritis. Mr. Powell was of the same opinion, but, owing to the extensive involvement of Littre's glands, desired confirmation. Cultures of the secretion gave pure *Staphylococcus albus*, even after

CHART XIV.—CASE OF CHRONIC GLEET, EXHIBITING PROLONGED NEGATIVE PHASES.



This case was one of chronic gleet of seven years' standing. Slight exacerbations were frequent. The chart presents several points of interest. The gonococic index was very low—0.35—indicating exhaustion of the protective mechanism. Despite this, splendid response was made to the first injection, the index rising in fourteen days to 1.8, to fall as rapidly to 0.7. The second injection, of like amount to the first, produced a much less satisfactory result, the index being yet lower after ten days; upon the sixteenth day it had recovered to 1.1. A third injection was then given. Upon the tenth day the index had fallen to 0.25, but during this interval the clinical symptoms had altered little. The index then began to rise rapidly, and continued to do so for twenty-six days; as the limit of rise seemed about reached, a fourth injection was then given of 125,000,000 organisms. Study of the curve between the third and fourth injections shows clearly that the third was given prematurely. The rise after the first injection and the subsequent fall were both so rapid that I wished to avoid a similar fall after the second injection; hence the mistake, which was avoided subsequently.

thorough irrigation of the urethra. The gonococcal index was found to be 0·9. The staphylococcal index was found to be 0·7. Confirmation was thus afforded of the non-gonococcal nature of the infection, which was considered to be staphylococcal in origin. A vaccine was made, and an injection of 150,000,000 organisms given. A second similar injection was given three weeks later, and the patient appeared to be improving. Unfortunately, he left England a fortnight later, and the success or otherwise of the treatment could not be determined.

Case 6 was a case of old gonococcal infection in the female, the discharge recurring at practically every menstrual period. Advantage was taken of the fact that during a period the index towards any infecting organism falls considerably: two days prior to menstruation the index was found to be 0·7; upon the fourth day it was only 0·3. The diagnosis of gonococcal infection was therefore made, and amply confirmed by the results of vaccine treatment. In three months the woman felt a totally different person, and had gained a stone in weight.

The evidence afforded by these and numerous other cases of chronic urethritis serves to indicate that in many instances, though the gonococcal infection has died out, the gonococcal toxin and antiseptics faultily applied have resulted in a weakened mucous surface, upon which numerous pathogenic organisms, usually of low virulence, are enabled to flourish and multiply. These prove extremely resistant in many instances to local forms of treatment, but sometimes readily respond to injections of vaccines, a series of which may, however, be required. In view of the extreme importance of eliminating every chance of the continuance of a gonococcal infection, it is good practice in all cases where the index is on the border-



line of the normal—*i.e.*, 0·8 or 1·2—to begin the treatment of such cases with injections of a gonococcal vaccine, even though no gonococci are to be found in the secretion. A first dose of 25,000,000 to 50,000,000 organisms is best given; should this produce no effect, it may be followed by a second of double the amount. Should only slight disturbance of the index result, the non-gonococcal nature of the infection may be considered established, and treatment then begun with a combined vaccine. Such treatment can do no possible harm, and may prevent a gonococcal case being missed. It must, however, be noted that even persistent treatment of some cases of chronic urethritis will fail to cure entirely the discharge and threads in the urine. The patients feel better, suffer no discomfort, and put on weight, yet a small bead of discharge may be expressed in the morning. Although such cases may safely be left in such a condition, none having retrogressed within my experience, further improvement may possibly be secured according to the method I employed in two instances. Both were very old chronic cases, one of thirteen years', the other of fifteen years' standing, and both when they came under my care had profuse discharge which worried them greatly. Every conceivable method of surgical treatment had been employed upon them. After a preliminary course of gonococcal vaccine, a whole series of vaccines prepared from the urethral organisms was employed in turn. Both cases improved very greatly, yet each morning it was possible to express a bead of discharge containing great numbers of organisms, both staining and failing to stain by Gram's method.

As a last resort I decided to inject cultures of living lactic acid bacteria into the urethra, with the aid of an



ordinary syringe, twice daily for a week. The first day there was slightly increased discharge and considerable itching; subsequently the injections resulted in less discharge, but the irritation continued. At the end of a week of this treatment the urethra was thoroughly flushed out with weak potassium permanganate solution night and morning. Next day the irritation had disappeared, and smears were obtained with great difficulty; no organisms could be detected, and cultures were also sterile. Both patients have remained well.<sup>1</sup>

It must not be thought that I under-estimate the value of proper surgical treatment in these refractory cases; gentle methodical dilatation, the topical application of antiseptics to infected glands and lacunæ, and flushing the canal with weak lotions, still remain the first essentials to success, but in vaccine treatment they may find a most useful adjuvant. In cases where the patient's means do not permit the employment of a surgeon skilled in the delicate manipulation of urethral work, or where such assistance is not procurable, reliance may be placed upon vaccine treatment combined with methodical douching of the urethra with a weak antiseptic. As always the combined method of attack will yield the speedier and probably more satisfactory result.

Loxton<sup>2</sup> entirely confirms my results in chronic gonorrhœa, his initial dose being 50,000,000.

#### ACUTE GONORRHOEA.

Three years ago I advocated the treatment of early gonorrhœa by means of a vaccine; subsequent experience

<sup>1</sup> The fluid employed for injection can be obtained from W. H. Martindale under the name of 'Trilactine liquid, special, for injection.'

<sup>2</sup> *British Medical Journal*, February 27, 1909.

has failed to modify this view. A first dose of 25,000,000 may be given as soon as possible in the course of the disease ; the second of 25,000,000 to 50,000,000 seven to ten days later. The necessity of preventing access of other pathogenic organisms can hardly be too strongly insisted on, and is best secured by means of a roll of cyanide of mercury gauze placed over the extremity of the penis ; flushing of the urethra with very weak antiseptic lotion by means of an irrigator twice daily at the appropriate period in the attack will greatly assist the cure, which is often complete within two to three weeks. If this be not so, then the dosage may be gradually increased to 75,000,000, 125,000,000, 250,000,000, at intervals of seven to ten days ; rarely an ultimate dosage of 500,000,000, or even 1,000,000,000, may be requisite. Control by means of the opsonic index is quite unnecessary ; clinical symptoms and microscopical examination of the secretion afford sufficient guide.

Three years' experience of this procedure has been altogether favourable to it : convalescence has been complete, as a rule, in about three weeks, while secondary complications and backward extension have failed to appear in any of the series. Although, when the patient's own vaccine has not been procurable, I have always made use of one which was highly polyvalent, prepared from first subcultures, and therefore presumably possessing high immunizing properties, the severe reaction reported by some observers has never been met with ; this, I think, is partly due to the low temperature (54° to 56° C.) employed in sterilization of the vaccine. Eyre<sup>1</sup> mentions hearing of three cases where injections of 25,000,000 to 50,000,000 were followed within forty-eight hours in one case by acute

<sup>1</sup> *Lancet*, July 10, 1909, p. 76.

orchitis, in the two others by joint involvement. These sequelæ, in my opinion, were in all probability entirely independent of the vaccine treatment ; for although such complications occur in but a small percentage of cases of acute gonorrhœa, yet sooner or later they are bound to be encountered. A possibility is that, had larger doses of vaccine been employed, these complications would have been obviated.

Nowhere is it more difficult to judge of results than in the case of gonococcal infections, for nowhere does natural immunity vary more ; to dogmatize, therefore, were foolish, but at the same time I cannot refrain from expressing the opinion that doses of 1,000,000 to 10,000,000 are entirely ineffective in influencing the course of a gonococcal infection. If one feels obliged to, begin with 5,000,000 to 10,000,000 ; but if good fail to come of it, push the dosage, and keep on pushing it till good does result, for never have I seen any ill effect.

#### GNOCOCAL ARTHRITIS.

What I have just said about deduction of a good result, and concerning dosage in cases of acute, subacute, and chronic gonorrhœa, holds with equal, if not greater, force in the case of this manifestation, so notorious for its intermissions and variable course.

The most complete experiments have been made in America by Cole and Meakins,<sup>1</sup> and by Irons.<sup>2</sup> Cole and Meakins, in fifteen cases, found the index subnormal, varying between 0·2 and 0·7. An initial dose of 200,000,000 to 300,000,000 was found to raise the index above normal in each case, the maximum height being at-

<sup>1</sup> Bull. of Johns Hopkins Hospital, June and July, 1907, p. 223.

<sup>2</sup> *Archives of Internal Medicine*, vol. i., No. 4, p. 433.

tained between the second and seventh day, while a return to normal occurred on about the tenth day. The injections were carried out under the guidance of the index (as estimated upon counts of fifty cells), and gradually raised till 1,000,000,000 were administered. General constitutional disturbance was very rare, and in only one case severe. The interval between injection was seven to ten days. They found that any coincident urethritis or prostatitis was much less amenable to the vaccine therapy than the arthritic condition. In regard to this latter they concluded that the results in the chronic cases were more marked than in the acute; cases which had progressed but very slowly under other methods of treatment showed much more rapid improvement. Their opinion was that the vaccine treatment had been of distinct value, a conclusion amply borne out by the clinical details given.

Irons<sup>1</sup> has also studied 40 cases of gonococcal infection, 31 being arthritic. In 15 of the cases the index was systematically studied, and found initially to be low, but clinical symptoms were used as the guide to size and interval of injections. The vaccines used were heterologous, and univalent, divalent, or trivalent, little advantage being noticed from the use of a polyvalent vaccine. At first initial doses of 20,000,000 to 50,000,000 were employed, but in later cases these were increased advantageously to 100,000,000, and even 1,000,000,000, the intervals between injections varying from three to seven days. The injection of 500,000,000 dead gonococci into the tissues of a person free from gonococcal infection was found in eight cases to produce practically no constitutional disturbance; quite otherwise was the result in infected cases. In these, within twenty-four hours,

<sup>1</sup> *Archives of Internal Medicine*, vol. i., No. 4, p. 433.

corresponding to the negative phase, there was increased articular pain, tenderness, rise in temperature, and general malaise, so that the suggestion was made to employ this reaction as a diagnostic in cases of doubtful gonococcal infection.

Inasmuch as the clinical course of gonococcal infections is very variable, and the great majority of arthritic cases ultimately recover spontaneously, Irons is particularly guarded in drawing conclusions from the results of his observations, but considers that in certain cases of gonococcal arthritis recovery can be hastened by vaccine therapy, this assistance being more marked in subacute and chronic ambulatory cases than in acute ones, although in several such cases improvement was apparently more rapid immediately following an injection than it was before.

#### GONORRHEAL VULVO-VAGINITIS IN CHILDREN.

Butler and Long<sup>1</sup> studied the effect of vaccine therapy in twelve such cases. They controlled their work by means of index determination, and reinjected before the index fell again below normal. They found that the dosage could only be determined by investigating each individual case, either a too small or a too large dose resulting in little or no response. The doses varied from 1,000,000 to 50,000,000, and an initial one of 5,000,000 is recommended.

The ages of the twelve cases were between one and a half and twelve years, and the results were compared with those obtained in twelve other similar cases treated locally with potassium permanganate and argyrol.

In four of the twelve cases treated with vaccine, clinical

<sup>1</sup> *Journal of American Medical Association*, March 7, 1908, p. 744.

evidences of gonorrhœa disappeared in from ten to twenty-one days, and gonococci could not be found in smears.

In five cases a cessation of discharge and disappearance of gonococci from the smears were secured after several weeks of treatment, a change from a univalent to a polyvalent vaccine being found beneficial.

Of the remaining three cases, in two the discharge ceased, but recurred after treatment was stopped, although ultimately gonococci disappeared from smears ; in the last case, recurrence was followed by cure.

Of the 12 control cases, in 9 treated respectively twenty - five, twenty - six, twenty - six, twenty - seven, twenty-nine, forty, forty-nine, sixty-three, and ninety-six days, cessation of discharge was not secured, while in the remaining 3, under treatment respectively thirty-one, thirty-two, and one hundred and seventy-six days, a favourable result was secured.

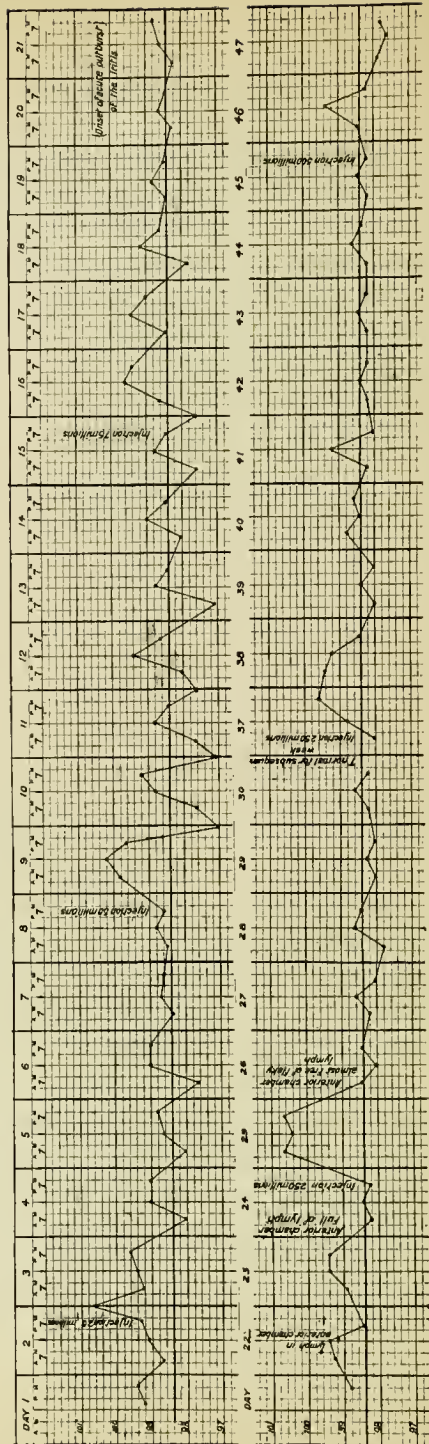
Their conclusion was that vaccine therapy appears to be far more efficient, and at the same time scientifically more tenable, than local antiseptic treatment in these cases.

Despite the researches of Torrey,<sup>1</sup> who, from a study of the agglutinins and precipitins in anti-gonococcal sera, came to the conclusion that the family gonococcus is heterogeneous rather than homogeneous, the view of its being a definite entity is usually held. Wherever possible, it is undoubtedly best to prepare a vaccine from the patient's own organisms ; but should the virulence of these have been reduced by antiseptic treatment or by the long duration of the infection, it is decidedly better to employ a vaccine made from strains of known high virulence. In eye cases one should inject immediately

<sup>1</sup> *Journal of Medical Research*, Boston, May, 1907, p. 329.



CHART XV.—To ILLUSTRATE THE CONDUCT OF A COURSE OF THERAPEUTIC INOCULATIONS UNDER GUIDANCE OF THE TEMPERATURE  
IN A CASE OF COMPLICATED GONOCOCCAL INFECTION. (For description see p. 139 *et seq.*)





the diagnosis is established, without waiting to determine the index or prepare a vaccine. The index in all eye infections, acute or chronic, due to whatever organism, is as a rule exceptionally high. The reason for this is fairly obvious. The circulation of that part is poor, the area of infection small ; consequently the toxins formed are absorbed in such minute quantities that they act like very small doses of vaccine, and tend to raise the index. If other areas in the body are infected, as is often the case in tubercular cases, this reasoning does not apply, and the index corresponds to the nature of the other area of infection. That the already high index so often fails to effect cure in these cases is due to the same cause—poor blood-supply and poor lymph flow ; hence in such cases high index is no contra-indication to injection.

As an instance of the management of a complicated case of gonococcal infection without the guidance of the opsonic index, which I now consider to be quite needless, I cannot do better than give brief details of the following case :

#### CASE WITH ILLUSTRATIVE CHART (No. XV.).

The patient was a male aged thirty-three. He had had three attacks of acute gonorrhœa, fourteen, eight, and two years prior to the present trouble. The last attack was complicated by gonorrhœal rheumatism in the knees, back, shoulder, and other joints, and from this little relief had been obtained by the various measures employed, which included a course of baths at Harrogate. In February, 1909, there was an attack of syphilis, for which a full course of rubbings was performed at Aachen, at the end of which the Wassermann test gave a negative reaction on two occasions. Prior to coming under my care he had

had several slight attacks of iritis and more or less continuous rheumatism, for which he had been taking aspirin regularly, without much result other than that of completely upsetting the digestive system. When I saw him early in October, 1909, the following symptoms presented themselves: Chronic urethritis with considerably enlarged prostate and seminal vesicles; subacute iritis in the right eye, with old adhesions in the left eye; multiple arthritis, especially bad in the case of lumbar and cervical spine, one knee, and both ankles. A few days' rest at home, where mild irrigation of the urethra was performed, and the pupils dilated, culminated in an exacerbation of the iritis and acute prostatitis, whereupon the patient was removed to a nursing home. Here complete rest was secured, and local and constitutional treatment directed against the iritis, arthritis, and urethritis. In addition, a course of vaccine treatment was decided on; but a formidable difficulty arose in connection with the dosage to be employed. Previous experience indicated an initial dose of 10,000,000 to 25,000,000 for the prostatitis, 50,000,000 to 75,000,000 for the urethritis, 250,000,000 for the iritis, and 500,000,000 for the arthritis.

As the arthritis was no worse than it had been, the iritis not very acute, but the prostatic pain severe, an initial dose of 25,000,000 was employed. The effect upon the temperature is to be seen in the chart (*vide* days 2 and 3). As the combined method of attack had produced a decidedly beneficial effect upon the prostatic and urethral condition, and the temperature was steady on the seventh day, a dose of 50,000,000 was injected upon the eighth day. This produced a marked effect upon the temperature, which continued to oscillate for six days. Upon the following day a dose of 75,000,000 was em-

ployed, with less disturbance of the temperature than on the preceding occasion, and upon the fourth day it was practically normal. Progress then seemed satisfactory; the urethritis and prostatitis were certainly better, the joints rather less swollen and painful, the iritis quieter. Suddenly, upon the following day, there was marked exacerbation of the iritis. For this Mr. G. Brookbanks James prescribed active leeching, hot-packs, diaphoretic drinks, strong solution of atropine locally, and mercury internally. Despite these measures, the anterior chamber three days later was full of flaky lymph, and the pain almost uncontrollable by injections of half-grain doses of morphia. The condition was rendered additionally serious by the fact that we could not be sure how much damage had been done to the other eye by previous subacute attacks. I therefore thought it well to remember my own dictum, that in gonococcal infection of the eye a dosage of 250,000,000 is the minimum of utility. This was accordingly administered, with some misgivings and no little fear of a resultant prostatic abscess. A marked reaction of the temperature followed, but after thirty-six hours the latter was normal and the anterior chamber nearly free from lymph. To what precisely this dramatic effect was due, it is of course impossible to say. The iritis was a very bad one, Mr. James declaring that he had only seen one or two worse. It had developed despite active local treatment, and disappeared within two days of the injection. For this, both Mr. James and myself could not help feeling that the vaccine treatment was largely responsible. The temperature remained practically normal, and no recrudescence occurred. The subsequent history of the case concerns itself with the treatment of the urethral condition, by Mr. Wyndham Powell, and

attention to the arthritic symptoms, which had remained little affected by the dosage already employed. It is the experience of all who have worked much on this condition, that doses of less than 500,000,000 organisms are almost certainly ineffective, and such proved to be the case here. Accordingly, on the thirty-seventh day the dose of 250,000,000 was repeated. The temperature speedily approximated the normal, and remained so, with the exception of a slight rise on the forty-first day. On the forty-fifth day the dosage was increased to 500,000,000, with very slight disturbance of the temperature, indication that even this dosage was provocative of but slight constitutional disturbance. Subsequently doses of 500,000,000 and 1,000,000,000 were employed at intervals of about eight days. The patient then left England for a warmer and drier climate for a few months, and his arthritis has steadily continued to improve.

The points upon which stress may be laid are briefly these : (1) That in such cases as these therapeutic immunization can be satisfactorily carried out under guidance of temperature, pulse, and general condition ; (2) that doses should be employed of such magnitude as will cause a rise in temperature of about  $1^{\circ}$  to  $1.5^{\circ}$  F. ; (3) that fresh inoculation should be deferred until the temperature has steadied itself for two to three days, or until it tends to rise again ; (4) that no arbitrary scheme of dosage for a gonococcal infection as such can be laid down. The locality is a most important point ; thus, for an acute prostatitis an initial dose of 25,000,000 is ample, whilst for an acute conjunctivitis or iritis one of 250,000,000 is obligatory, a dose which in turn will probably have no beneficial effect upon a chronic arthritic condition.

## CHAPTER IX

### THE COLON, TYPHOID, AND DYSENTERY GROUPS

THE investigations of Gaertner, Achard and Bensaude,<sup>1</sup> Gwyn, Schottmuller,<sup>2</sup> Kurth, Brion and Kayser,<sup>3</sup> and others, have revealed the existence of several organisms which occupy an intermediate position between the *Bacillus coli communis* on the one hand, and the *Bacillus typhosus* on the other. Some of these, such as Gaertner's bacillus, in their characteristics the more nearly resemble the colon bacillus, and are therefore known as 'paracolon bacilli'; others more nearly resemble the *Bacillus typhosus*, and are known as 'paratyphoid bacilli' (*A* of Brion and Kayser, *B* of Schottmuller). In addition to these are the various members of the dysentery group (Shiga, Flexner, Hiss, Kruse, Strong).

The fact that these intermediates are capable of causing lesions in the human subject has now been well established,<sup>4</sup> and causes them to assume an added importance.

The differentiation of the various members of this group the one from the other is by no means easy, and hardly comes within the scope of this small book. Suffice to say it depends chiefly upon agglutination experiments

<sup>1</sup> *Bull. Soc. Méd. des Hôp. de Paris*, 1896.

<sup>2</sup> *Zeitschr. f. Hyg. u. Inf. Bad.*, xxxvi., 1900.

<sup>3</sup> *Münch. Med. Woch.*, 1902, p. 611.

<sup>4</sup> For two fatal cases of infection by Gaertner's bacillus in infants, see Batten, Proceedings of the Royal Society of Medicine, March, 1908, p. 81.

and the fermentative reactions in various carbohydrate media. For convenience, the colon, typhoid, and dysentery groups will be described as if sharply defined from each other. The pathogenic effects of all three appear to be due almost entirely to endotoxin, which produces both a local and general reaction; a hæmolysin is also formed to a greater or less extent in the case of the colon dysentery bacilli.

In the production of immunity, both bacteriolysis and phagocytosis play a part—the latter to a much greater extent than was formerly supposed. Probably it is the more important factor, inasmuch as these bacteria set free their endotoxin when treated with bacteriolytic sera.

In the preparation of the vaccines of these organisms, autolysis occurs with the liberation of the endotoxin. A toxic effect is therefore produced by their injection, and for this reason the initial dose should not be an excessive one. If no heat be employed for sterilization purposes, less autolysis occurs, and therefore less constitutional disturbance is produced. Other advantages there are, which will be referred to later (*vide* p. 155).

### I. THE *BACILLUS COLI COMMUNIS* GROUP.

The *Bacillus coli communis* and its near allies are especially associated with disease of the abdominal organisms, either alone or in conjunction with some other bacterium, such as the streptococcus, staphylococcus, pneumococcus, *Micrococcus tetragenus*, or *Bacillus pyocyaneus*. Among the conditions set up which are more or less amenable to vaccine therapy are peritonitis, cystitis, urethritis, pyelitis and pyelonephritis, endometritis, enteritis, perityphlitis, cholecystitis, subphrenic



and hepatic abscess, and even empyema and suppurative periostitis.

For the successful vaccine treatment of these conditions, several things must be carefully borne in mind, among which are—

1. The fact, already mentioned, that the term *Bacillus coli* is a generic one. Thus, Glenn<sup>1</sup> points out that other organisms, such as *Bacillus cloacæ*, which very closely resemble the *Bacillus coli*, may produce cystitis. The author himself has several times isolated bacilli from the urine in cases of bacilluria which were atypical. Thus, in one instance the micro-organism resembled *Bacillus coli* in every detail except that it was rather positive than negative to Gram's stain, and failed to ferment certain carbohydrate media. The necessity of employing the autogenous vaccine, whenever possible, and in default of this a highly polyvalent vaccine, is therefore obvious.

2. That not only is the vaccine, especially if heat be employed to secure sterility, highly toxic, but also that sinuses due to this organism show a marked tendency to close—a tendency which must carefully be counteracted, or rigors and other signs of auto-intoxication will ensue. Where this is likely to occur, an initial dose of 25,000,000 should not be exceeded. Later, very much higher doses, even to 1,000,000,000, may be safely employed.

3. It would appear that, after an autogenous vaccine has been employed for six to eight weeks, the living bacteria at the site of infection occasionally manifest the power of elaborating a protective substance against the opsonin. This difficulty may be surmounted by taking fresh cultures and preparing a new vaccine.

<sup>1</sup> *Journal of Infectious Diseases*, June 12, 1909, p. 339.



4. The discharge of bacilli in cases of bacilluria may be markedly intermittent. It is never safe, therefore, to assume that infection has disappeared because one or two examinations of the urine point in this direction. Such examinations must be repeated at intervals of about three days for at least as many weeks. In this connection it must be remembered that certain cases which appear to be merely cystitis are in reality ones of renal disease, and may be complicated by tuberculosis; while bacilluria may merely be evidence of colon infection elsewhere in the abdomen, as of the bowel, uterus, or old caseous abdominal glands.

If due regard be paid to these important points, it may be said that the prognosis is almost uniformly favourable in sinus cases, whether coming from the region of the liver and gall-bladder, appendix, pleura, or bone, provided that the appropriate surgical measures can be also pursued.

As to the resultant benefit in cases of bacilluria, diametrically opposite opinions have been expressed. Thus, Geraghty<sup>1</sup> came to the conclusion that four cases derived not the slightest good; Davis,<sup>2</sup> from the effect in eight cases, expresses a favourable opinion; while Wright, Western, Eyre, Turton, and others, have met with more successes than failures. Charlton Briscoe<sup>3</sup> advises in colon bacilluria that the bowel should first be thoroughly evacuated with the aid of calomel, the urine alkalinized, and five- to ten-grain doses of urotropin given. If these measures fail, then a vaccine should be tried, in initial doses of 25,000,000 to 50,000,000. Emery<sup>4</sup> gives as his experience of coli cystitis: 'Vaccine treat-

<sup>1</sup> *Johns Hopkins Hospital Bulletin*, January, 1909, p. 12.

<sup>2</sup> *Journal of Infectious Diseases*, April 1, 1909, p. 224.

<sup>3</sup> *Lancet*, October 30, 1909, p. 1269.

<sup>4</sup> 'Immunity and Specific Therapy,' pp. 394, 395.

ment cures all the symptoms, and reduces the pus and bacilli present in the urine to a small fraction of its original amount, but fails to remove them entirely. There is no doubt that the treatment is the best available.'

In acute nephritis, and especially in the pyelitis of pregnancy, recourse should be taken to vaccine treatment when colon bacilluria is present. It is, of course, well known that a bacteriuria tends speedily to clear up after evacuation of the pregnant uterus; but when acute symptoms appear in the third or fourth month, it is better not to await delivery, but to employ a vaccine—a method of treatment which has yielded most successful results at the hands of some.

Hale White and Eyre<sup>1</sup> give details of rapid and complete recovery of a severe case of cystitis and double pyelitis, accompanied by vomiting, pyrexia, and high pulse. An initial dose of 5,000,000 of a vaccine of her own *Bacillus coli* resulted in immediate improvement, was followed in four days by a dose of 30,000,000, and ten days later by one of 250,000,000. A year later there had been no return of her trouble. Other cases, including a somewhat remarkable digestive case (No. XI.), will be found described in the same paper.

Butler Harris<sup>2</sup> draws attention to the interesting fact that in many cases of slight endometritis with cervical catarrh the colon bacillus is present, and fall in the index to this organism is coincident with depression of the local and general condition. He finds that 5,000,000 of a vaccine given a week after the period, and repeated a week before the next, will cure the local infection and improve the general health. The treatment has, how-

<sup>1</sup> Proceedings of the Royal Society of Medicine, June, 1909, p. 146.

<sup>2</sup> *Practitioner*, May, 1908, p. 647.

ever, to be continued for a considerable time—perhaps six months. In two cases, such as he describes above, I have had the opportunity of taking smears and cultures from the cervix uteri. In both no coli bacilli were to be seen, the only organism which was present both intra- and extracellularly in large numbers being a very large Gram-positive bacillus, which on agar and blood-agar gave colonies indistinguishable from a streptococcus, and in broth gave a granular deposit and slight uniform turbidity; its sugar reactions distinguish it from the *Bacillus Caucasicum* or Bulgarian bacillus. Treatment has been begun with a vaccine of this organism, but it is too early to form any estimate of the result.

Butler Harris<sup>1</sup> also states that in mucous colitis he has obtained good results. This is confirmed by Hale White and Eyre in three cases, and by the author in two. It would appear that in vaccine treatment we are afforded a most useful adjuvant to the rather unsatisfactory methods of dealing with this distressing complaint; the pain is lessened, the diarrhoea, mucus, and blood diminished, and general health much improved.

In puerperal septicæmia, the success or otherwise of vaccine treatment depends almost entirely upon the accuracy of the diagnosis of the infecting organism or organisms. The commonest are: Streptococcus, which is usually of the 'mucosus' type, staphylococcus, pneumococcus, and *Bacillus coli*, either alone or in conjunction. Bonney<sup>2</sup> emphasizes the gravity of the occurrence of *Bacillus coli* in conjunction with either of the others. Care must be taken to discriminate between a case of true puerperal septicæmia, where the prognosis is

<sup>1</sup> *Practitioner*, May, 1908, p. 647.

<sup>2</sup> *Clinical Journal*, August 19, 1908.

serious, and one of localized infection of the uterus by the colon bacillus. Delay in the latter case while the results of treatment upon general lines are being awaited is excusable, in the former case unpardonable. Should blood-culture confirm the bacteriological findings in the uterine discharge, no hesitation should be felt in having recourse to vaccine treatment. The worse the condition of the patient, the greater the necessity for prompt action. Upon the reserve strength of the patient everything depends. If this be adequate to tide the patient over two or three days' treatment, the administration of the autogenous vaccine in 5,000,000 to 10,000,000 initial doses should secure recovery in practically every case. In twelve to eighteen hours the temperature should drop 2° to 3° F., the pulse and general condition improve. Any sharp rise of temperature, or a steady rise, however slight, continued over twelve hours, should be the signal for a fresh injection.

Wright and his co-workers have recently demonstrated the value of opsonic index determinations as a diagnostic aid in cases of supposed appendicular or other abdominal abscess. The advisability of raising the opsonic index to the colon bacillus prior to abdominal operations, in cases where contamination from the infected focus is feared, is worthy of earnest consideration. A dose of 25,000,000 to 50,000,000 one or two days prior to the operation will suffice.

The following case of infection by the *Bacillus coli communis* is not without interest. The patient was sent to me by Mr. Wyndham Powell, suffering from subacute urethritis, for treatment with a gonococcal vaccine. Four injections were given, the index being raised from 0.37 to 1.6. The urethral condition was decidedly improved, and the discharge practically ceased. Considerable dis-

comfort, however, was felt in the region of the prostate, and finally culminated in an attack of acute prostatitis and cystitis. Nothing definite could be felt per rectum. The colon bacillus was isolated from the urine, and 200,000,000 of an autogenous vaccine given. A rigor and severe general disturbance resulted. This would appear to be especially liable to happen in this infection, and to denote closure of a sinus or suppressed discharge, and re-examination per rectum revealed a small nodule in the prostate. Upon this breaking down and discharging, improvement began, and two injections, one of 100,000,000, the other, fourteen days later, of 150,000,000, resulted in cure.

## II. INFECTIONS BY THE TYPHOID GROUP OF BACILLI.

The most important members of this group are the *Bacillus typhosus abdominalis* (Eberth), the *Bacillus paratyphosus A* (Brion and Kayser), and *Paratyphosus B* (Schottmuller). Other slightly variant forms have also been described. The practical importance of the discovery of these different members is very great, and especially with regard to the question of the production of antityphoid immunity. Epidemics of pseudo-typhoid fever have been described from Germany,<sup>1</sup> France, and America, and isolated cases have been reported in Great Britain, India, etc. The paratyphoid fevers have occurred in series of true enteric fever, in house epidemics, and under circumstances which point to the disease being sometimes water-borne.

The clinical features have been very variable, but three types may be described :

<sup>1</sup> Hunerman, *Zeitschrift f. Hyg. u. Infect. Krankh.*, 1902, Bd. xl., p. 522; and Schottmuller.

*Type I.* closely resembles mild typhoid fever, and can only be distinguished by failure of the blood-serum to agglutinate the *Bacillus typhosus*, and its power to agglutinate one of the other organisms of the group. As these cases all recover, the anatomical lesions are unknown. Hæmorrhage, phlebitis, and relapse, have been described as complications.

*Type II.* presents the clinical features of septic infection, and resembles the so-called typhoid septicæmia, or enteric fever with intercurrent or terminal sepsis. These sometimes end fatally. Enlargement of the spleen is a constant feature; sometimes the intestines are normal, at other times ulcerated, but the ulcers are like those due to dysentery rather than to typhoid, the solitary follicles, Peyer's patches, and mesenteric glands being as a rule unaffected.

In both Types I. and II. rose spots and sore throat are very prominent features.

*Type III.*—In these cases the organisms have been found in abscesses in patients in whom no history of enteric fever was obtainable.

Both in true enteric and in the fevers of Types I. and II. the organisms can be almost invariably isolated from the blood according to the following method: The medium employed is ox bile, 90 c.c.; glycerine, 10 c.c.; peptone, 2 c.c. Twenty c.c. of this are put into small flasks and sterilized. About 9 c.c. of blood is then withdrawn from a vein in the antecubital fossa, with aseptic precautions, and 3 c.c. run into each of three flasks, which are then incubated for twelve to twenty-four hours. Stroke cultures are then made on a litmus lactose agar plate, upon which growth may often be seen in six hours.



Coleman and Buxton<sup>1</sup> employed this method in a large series of cases, and isolated the organism in every instance before the second week of illness, and often before the serum reaction developed. After the second week there are much fewer organisms in the blood. Ninety per cent. of thirty-three relapses also gave a positive result.

Failing to isolate the organisms by blood-culture, it remains to attempt their isolation from the urine and fæces according to the approved methods.

Having obtained a culture, test is then made for the Widal reaction, both with the organism or organisms and with a standard strain of the *Bacillus typhosus*. For it must not be forgotten that the patient may be infected with both the *Bacillus typhosus* and *Paratyphosus*.<sup>2</sup>

In Europe, the *Paratyphosus B* appears to be the commoner. In India, Semple<sup>3</sup> found the *Paratyphosus A* in four cases, the *Paratyphosus B* in two cases.

Proescher and Roddy<sup>4</sup> report the occurrence of forty-eight cases due to the *B. paratyphosus A* in the Allegheny Hospital in 1907-08, and Statham<sup>5</sup> of four cases in Pretoria due to the same bacillus.

### *Antityphoid Immunization.*

Of the bearing of these results upon the statistical side of therapeutical immunization against enteric fever sight must not be lost. As to the merits or demerits of the procedure, considerable diversity of opinion has existed. The latest statistics, and especially those recorded by

<sup>1</sup> *American Journal of Medical Science*, June, 1907.

<sup>2</sup> For such a case see *Scientific Memoirs of the Medical Department of Government of India*, No. 32, 1908, p. 31.

<sup>3</sup> *Ibid.*

<sup>4</sup> *Journal of American Medical Association*, February, 1909.

<sup>5</sup> *Journal of Royal Army Medical Corps*, October, 1908, p. 356.



Luxmore<sup>1</sup> in the case of the 17th Lancers, and by Firth<sup>2</sup> and by Leishman,<sup>3</sup> are much more favourable ; but before any definite conclusions can be formed it is obviously necessary to take into account the possibility of a given epidemic, or of a certain number of cases in such an epidemic being really due to one or other of the paratyphoid bacilli, and to this hitherto no attention has been paid. The advisability of employing a vaccine containing not only the *Bacillus typhosus*, but also the several paratyphoid bacteria, is worthy of consideration.<sup>4</sup>

*Preparation of Antityphoid Vaccine : its Effects and Method of Use.*

The mode of preparation approved by the Army Council is as follows : A non-virulent strain is grown on broth of a definite reaction, and incubated at 37° C. for twenty-four to forty-eight hours. In order to encourage free growth special flasks are used, giving a shallow layer of about 1 inch of medium, so as to permit of good aeration, this being essential to obtain maximum development. The contents of the flask are then standardized in the usual way, and sterilized by heating on a water-bath for one hour at 53° C. Sterility is proved by aerobic and anaerobic cultures, and 0·25 per cent. lysol added. The vaccine is diluted if necessary, so as to contain 1,000,000,000 bacteria per c.c., and put up in glass bulbs containing 1 c.c. and  $\frac{1}{2}$  c.c. respectively.

To insure antityphoid immunity the smaller dose is

<sup>1</sup> *Journal of Royal Army Medical Corps*, January to June, 1907, p. 492.

<sup>2</sup> 'Military Hygiene,' 1908, p. 64.

<sup>3</sup> *Journal of Royal Army Medical Corps*, February, 1909, p. 163.

<sup>4</sup> A vaccine of this composition can now be obtained from W. H. Martindale, 10, New Cavendish Street.

injected into the flank or at a spot about 1 inch below the centre of the clavicle ; if convenient, the patient may go to bed for twenty-four hours, as sometimes considerable constitutional disturbance results. Some degree of malaise nearly always is produced. In a small percentage of cases there is a tendency to faintness, and occasionally a rigor occurs between the first and sixth hour. There is a certain amount of pyrexia, usually about  $101^{\circ}$ , rarely as high as  $103^{\circ}$ , which generally passes off completely at the end of twenty-four hours, though occasionally it persists for another twenty-four hours.

The local effects are as follows : Tenderness begins to make itself felt in five or six hours, and is worst at about eighteen hours, when there is redness over an area of 4 or 5 square inches. Occasionally the lines of the lymphatics may be traced, and slight tenderness is felt in the armpits. These local symptoms should practically pass away in forty-eight hours. Ten days later the larger dose is given, and should produce no ill effects.

In place of the method of preparation given above, Castellani advocates the following : Two loopfuls of a twenty-four hours' agar culture of an avirulent strain are introduced into 10 c.c. of broth, and incubated for twenty-four hours. The broth is then heated to  $50^{\circ}$  C. for half an hour. This leaves the bacteria, or at all events some of them, still alive. Of this 0.5 c.c., 1 c.c., 1.5 c.c., are given successively at intervals of a week. Local reaction and some malaise ensue—certainly with the first and second injections, but probably not with the third. Castellani claims that by this method a much higher degree of immunity is produced.

The ill effects upon the vaccine of excessive heat have been demonstrated by Harrison, Leishman, Semple and

Matson. Thus, Harrison finds that heating to 60° C. deprives the vaccine of much of its immunizing power ; Leishman, that a vaccine treated at 53° C. possesses marked properties for only three months ; while Semple and Matson<sup>1</sup> have shown that complete sterility and high immunizing power is secured by the mere addition of carbolic acid to 0·5 per cent. After twenty-four hours the bacteria are all dead, and full powers are possessed for at least two years.

The effects of the inoculation of such a vaccine have now been fully studied. In estimating the typho-opsonic index the sera are diluted to 1 in 5, in order to diminish bacteriolysis. The opsonic and phagocytic indices<sup>2</sup> appear to be depressed for three to six weeks after commencing treatment, during which interval exposure to infection has an added danger ; it then rises above normal. There is also a marked rise of four to six times in the bactericidal power.

The duration of the immunity thus conferred has been studied by Harrison.<sup>3</sup> He found that evidence of a bactericidal activity higher than normal and of agglutinins could be obtained from the serum of men who had been inoculated as long as six years previously ; but whether the protection that so remains will still suffice to ward off an attack of enteric fever is not yet known.

The rôle of agglutinins and bactericidal substances in the production of typhoid immunity is unsettled. Thus, Jorgensen<sup>4</sup> found that the agglutinating power usually declines after the third week, and that a high agglutinative power does not appear to protect against

<sup>1</sup> *Lancet*, August 14, 1909, p. 436.

<sup>2</sup> Harrison, *Journal of Royal Army Medical Corps*, May, 1907, p. 472.

<sup>3</sup> *Ibid.*

<sup>4</sup> *Centralb. f. Bakt. u. Parasit.*, Jena, 1908, Bd. xxxviii., p. 475.

relapse or recurrence, and the same is possibly true of the bactericidal substances ; while Stern and Korte<sup>1</sup> found in a patient, whose serum revealed the highest bactericidal power ever observed by them, that a relapse developed eight days later. A possible fallacy in these observations lies in the fact that they were conducted *in vitro*. Topfer and Jaffe<sup>2</sup> found with the Pfeiffer method *in vivo* that the serum of convalescents was more effective bactericidally than that of acutely ill typhoid patients ; while Klein,<sup>3</sup> and Neufeld and Kuhne,<sup>4</sup> have found increase of opsonin, and especially of specific opsonin, in the 'immune' sera of convalescent cases.

*The treatment of enteric fever with specific sera, filtrates, and residues*, has been studied in a series of 204 patients by Richardson.<sup>5</sup>

Of these 204 cases, 74 underwent ordinary routine treatment ; 35 routine treatment, together with the sera of immunized horses given in various ways ; 74 routine treatment, combined with subcutaneous injections of about 3 c.c. daily of a sterile filtrate of bouillon cultures ; 21 routine treatment, combined with daily subcutaneous doses of 2 to 10 c.c. of typhoid vaccine.

The administration of these immunizing agents was uncontrolled by index determinations.

His conclusions are most guarded, but are as follows : That, despite the handicap to specific therapy in the difficulty of early diagnosis—

1. Specific therapy, confined to the original disease, increases, apparently, the tendency to relapse.

<sup>1</sup> *Berlin. Klin. Woch.*, 1904, Bd. xli.

<sup>2</sup> *Zeitschrift f. Hyg. u. Infect.*, Leipzig, 1906, Bd. lii., p. 393.

<sup>3</sup> *Johns Hopkins Hospital Bulletin*, June and July, 1907, p. 245.

<sup>4</sup> *Arb. a. d. k. Gesundheitsamte*, Berlin, Bd. xxv., p. 164.

<sup>5</sup> *Boston Medical and Surgical Journal*, vol. cvii., No. 14, p. 449.

2. Inoculation with typhoid vaccine continued into convalescence largely eliminates the risk of relapse.

3. Antityphoid serum is no more effective than filtrates or vaccine, and is much more expensive.

4. Typhoid filtrates may exert a powerful effect upon the clinical course, their use being followed in many instances by chills or rise in pulse and temperature, these being often followed in their turn by marked fall in pulse-rate and temperature, and a general improvement in the clinical picture.

5. That a vaccine seems to make the typhoid process longer, but milder, and is apparently very effective in the prevention of relapses.

Walters and Eaton<sup>1</sup> record their results in thirty-four cases of typhoid fever treated with vaccines. The mortality among these equalled 3·2 per cent., as compared with one of 11 per cent. in forty-six cases treated in the ordinary manner. They concluded—

1. That an early administration of vaccine materially shortens the duration.

2. That even in those cases where the temperature is not favourably influenced the clinical symptoms seem to show distinct improvement, the disease running a mild course.

The patients declared that they felt better very soon after the initial dose, which may be 50,000,000 to 100,000,000, and is to be repeated every two to three days, or at each marked rise in temperature.

Semple<sup>2</sup> has treated nine cases with vaccine. He advises the administration of 15,000,000 to 30,000,000 daily for from four to six days in succession, a fresh site

<sup>1</sup> *Boston Medical and Surgical Journal*, April 22, 1909, p. 508.

<sup>2</sup> *Lancet*, June 12, 1909, p. 1668.

being employed upon each occasion. In default of the guide of the opsonic index he would employ the control of the Widal reaction and clinical signs. A stock vaccine should be used while the patient's own is being prepared. He finds this method devoid of risk. Whether the duration of the attack is shortened thereby he is unable to say, but in four of the nine cases this appeared to be the case. In six of the nine cases well-marked improvement set in after the first inoculation, especially in two where an autogenous vaccine was used. In the remaining three cases there was an amelioration of the clinical signs.

Smallman<sup>1</sup> treated thirty-six cases, with three deaths. Of these three, two were fulminating cases which died in a week, and only received one dose.

His initial dose of 250,000,000 was given in the forenoon ; by the evening there was a rise in temperature of a degree or two, corresponding to the negative phase. The following morning there was the usual fall, which is progressive for two or three days, when the dose should be repeated. The patients said they felt markedly better after injection, and presented an unexpectedly good appearance. The urine increases, perhaps to even double the amount. There was an unusual absence of prolonged cases, of complications, of sequelæ, and of relapses. In two cases there was commencing periostitis of the tibia. An injection of about 100,000,000 was given deeply over the lesion. In a few hours the pain suddenly disappeared, and swelling subsided. Smallman's impression from this small series was that the treatment held out considerable promise of good.

The following case of my own is not devoid of interest : The patient was a well-known athlete, aged thirty-eight.

<sup>1</sup> *Journal of Royal Army Medical Corps*, February, 1909, p. 136.



About three months previously he had contracted typhoid fever. Two relapses had occurred, and about a week prior to my seeing him infarction of the lung had occurred. His general condition was very unsatisfactory : pulse 120 to 140, weak and irregular ; temperature  $101^{\circ}$  to  $103^{\circ}$  F. ; mind wandering. His sputum was thick and blood-stained. Cultures yielded no pneumococci, only a few *Streptococcus mucosus*, and many *Bacillus typhosus*. Blood-cultures also yielded the latter bacillus. A vaccine had just been prepared, when a third relapse occurred, the temperature rising to  $105^{\circ}$  to  $106^{\circ}$  F., the pulse becoming very weak, rapid, and irregular, and pneumonic symptoms appearing in both lungs. A dose of 50,000,000 was at once given. The temperature at once began to fall. Within twenty-four hours it was sub-normal, and only once subsequently rose above  $99^{\circ}$  F. The pulse became stronger and more regular, and the mind more clear. Recovery was practically uninterrupted. The entire absence of pneumococci from the sputum, and the presence therein of the *Bacillus typhosus*, raises in my mind the question as to the frequency of this in cases of so-called 'typhoidal pneumonia.' I cannot help feeling that for most of these the *Bacillus typhosus* is alone responsible. The immediate good effect of one dose of vaccine in this case, which so obviously failed to immunize itself, was very striking.

Consideration of the favourable opinions of the results of vaccine treatment expressed by the above authorities will surely suggest to those of impartial mind the advisability of the routine treatment of typhoid fever by this method, combined, if thought advisable, with the administration of an anti-endotoxic serum, as recommended by Chantemesse and others.



*Typhoid Carriers.*

By a typhoid carrier is meant an individual who, without presenting any typhoidal symptoms, yet continues to excrete typhoid bacilli in urine or fæces, intermittently or otherwise, often over a space of many years. The first of such cases reported was by Houston,<sup>1</sup> though prior to this cases had been reported of abscesses due to the *Bacillus typhosus* many years after an attack of typhoid fever.

A careful study of recent epidemics has revealed the fact that in many instances these have been initiated by the contamination of food or water supply by the urine or fæces of old enteric patients who have exhibited no symptoms for many years.

Semple has carefully investigated several of these cases, and finds that the discharge of bacteria may be markedly intermittent, occurring at intervals perhaps of a month apart. He explains this occurrence as follows: The typhoid bacilli during an attack are deposited in the liver or kidney. In the former case they may infect the bile—a most favourable medium for their growth—and are poured into the intestine and excreted in the fæces. In the latter case an infected focus discharges at intervals and infects the urine. In confirmation of this view is the fact that he examined the bile in seventeen fatal cases of enteric, and found it infected in ten of these. These observations have been confirmed by Davies and Walker Hall,<sup>2</sup> Kayser and Klinger, and by others.

Graham, Overlander and Darley,<sup>3</sup> investigated the stools and urine of sixty-five patients within a week of

<sup>1</sup> *British Medical Journal*, January 14, 1899.

<sup>2</sup> *Lancet*, November 28, 1908.

<sup>3</sup> *Boston Medical and Surgical Journal*, January, 1909, p. 38.

their discharge from hospital as cured. In fifteen cases either the *Bacillus typhosus*, *Paratyphosus A* or *B* were found. What proportion of these would become true carriers it is impossible to say, but the statement has been made that about 5 per cent. of cases of typhoid fever become chronic carriers.

As a result of these investigations, the question of careful systematic testing of the urine and fæces of convalescent typhoid cases before their discharge becomes a very important one.

That vaccine treatment of these cases may bring about the disappearance of the bacilli is rendered hopeful by this result being apparently achieved in a case of Irwin and Houston.<sup>1</sup> A carrier of seven years' duration was excreting large numbers of bacilli in the urine, none in the fæces. An injection of 50,000,000 produced rise of temperature, headache, and malaise ; a dose of 100,000,000 eight days later produced less reaction, and was followed eighteen days later by one of 200,000,000. Two days after the third injection the urine was crowded with bacilli. It was then rendered of alkaline reaction by the administration of sodium lactate. Eighteen days later the *Bacillus typhosus* had disappeared from the urine, nor was it subsequently found. A dose of 300,000,000 was followed by one of 500,000,000 fourteen days later, and by one of 1,000,000,000 after twenty-nine days. Agglutination was now produced only in a dilution of 1 in 10, instead of in one of 1 in 200, as formerly. There was considerable improvement in the general health, and a gain of 10 pounds in weight. The patient is still being watched for a recurrence of the bacilluria.

<sup>1</sup> *Lancet*, January 30, 1909, p. 311.

## III. THE DYSENTERY GROUP.

Of the various forms of dysentery, the only one with which vaccine treatment is concerned is the bacillary. Several closely-allied bacteria, differing mainly in their sugar and agglutinative reactions, have been described in different epidemics by Shiga, Kruse, Flexner, Hiss, and Strong, and bear their respective names.

The chief work from the point of view of immunization by means of vaccines has been done by Captain W. H. C. Forster, of the Indian Medical Service. During the past few years he has been actively engaged upon the subject, and to his publications and letters I am indebted for the following account :

Clinically, bacillary dysentery may be divided as follows :

## CLASS I. Acute dysentery—

1. Gangrenous.
2. Non-gangrenous.

## CLASS II. Chronic dysentery, which may be subdivided into—

1. Cases of weeks' or months' duration, in which the patient is still passing dysenteric stools, either continuously or intermittently.
2. Cases of years' duration, in which the patient has ceased to pass dysenteric motions, and in whom the symptoms consist of flatulent diarrhœa, accompanied by abdominal pain of a peculiar type.

Of these, the following are suitable for vaccine therapy :

The non-gangrenous cases of Class I., which have

resisted treatment for seven days, and in which the patient is not obviously moribund.

Of Class II. all cases are suitable except those in whom the bowel is so extensively damaged by chronic ulceration as to render hopeless treatment of any sort.

Although the cases treated have been variously infected by the following different strains—Kruse-Shiga, Flexner, and Y. of Hiss—the vaccine employed has been prepared only from the Kruse-Shiga type ; so that the results achieved are the more striking. Uniformly satisfactory results have been obtained and recorded by Forster,<sup>1</sup> Gillitt,<sup>2</sup> Stephen,<sup>3</sup> Newman,<sup>4</sup> Castellani,<sup>5</sup> many of the cases being very striking, especially in the chronic relapsing ones, in which all medical treatment had been unavailing. Thus, of ten such chronic cases, Forster completely cured seven, these remaining perfectly well for twelve months after treatment.

In the Midnapore Gaol the case mortality over six years averaged 6·3 per cent. ; after the adoption of Forster's vaccine it fell to 0·9 per cent.

The scheme of treatment laid down by Forster is as follows : The immediate injection of acute as well as of chronic cases by means of a stock vaccine, given in small doses at fixed intervals, without the control of the opsonic index. Of course, common sense must be applied if clinical symptoms contra-indicate the repetition of a dose at a given time. In acute cases the commencement of vaccine therapy is contra-indicated from the fourth to the twenty-first day, during which interval the anti-endotoxic

<sup>1</sup> *Indian Medical Gazette*, June, 1907, p. 201.

<sup>2</sup> *Ibid.*, January, 1908, p. 12.

<sup>3</sup> *Ibid.*, October, 1907, p. 375.

<sup>4</sup> *Lancet*, May 16, 1908, p. 1410.

<sup>5</sup> *Archiv f. Schiff u. Trop. Hyg.*, Bd. xi., Heft 3.

sera of Kruse and Shiga may be given in full doses frequently repeated.

As different strains of the organisms vary greatly in their toxicity, and as toxic strains are liable to produce very violent local and general reaction, even in small doses, counting methods of standardization are not employed, but the strength of the vaccine is so regulated that the minimum lethal dose for a rabbit of 1,200 to 1,400 grammes in weight is not less than 0·4 c.c. Of such a vaccine his dosages are, for an adult, in both the acute and chronic forms—

First dose	..	..	..	..	0·1 c.c.
Second dose	..	..	..	..	0·2 c.c.
Third dose	..	..	..	..	0·3 c.c.
Fourth dose	..	..	..	..	0·4 c.c.

For females a slight reduction is necessary.

In the case of a child of nine, a first dose of 0·05 c.c. was given, and a second of 0·1 c.c., without ill effects and with a good result.

The doses indicated above produce practically no negative phase, and in fourteen days the immunity is more than a hundred times what it was before the dose.

Forster employs intervals of ten days between the doses, and usually proceeds to the fourth dose, to make sure that the patient has got rid of all bacilli and is not likely to become a chronic 'carrier.' If it be necessary to go beyond the fourth dose with increased quantities, the bowel symptoms must be carefully watched, as large doses are very toxic, and in animals, at all events, badly tolerated. In man, the symptoms of overdosage take the form of dysenteric pains in the bowel, with diarrhœa and even blood and mucus. Most doses are followed

by some slight symptoms of the sort, but if these are severe in any given case, a reduced dosage must be employed.

Vaccine treatment would appear to hold out promise of success in the case of summer diarrhœa of infants, and in asylum dysentery.

## CHAPTER X

### CATARRHS OF THE RESPIRATORY TRACT AND ITS ANNEXES—BRONCHITIS AND ASTHMA

THE importance of an exact bacteriological study of the causative factors in the production of simple catarrhal affections of the respiratory tract has become much more evident during the past two or three years, inasmuch as the connection between these and pneumonia, asthma, bronchitis, sinusitis of the accessory air-spaces, middle-ear affections, arthritis, septicæmia, and the secondary infections of pulmonary tuberculosis, has been definitely established. So extensive and complicated is this subject that space can be found for merely the briefest of résumés. Within the next year or two I hope to bring out a treatise devoted entirely to the bacterial affections of the respiratory tract.

Thanks to the pioneer work of Cantley,<sup>1</sup> Dunn and Gordon,<sup>2</sup> Bezançon and De Jong,<sup>3</sup> Prosser White,<sup>4</sup> Cardone, Benham,<sup>5</sup> and the author,<sup>6</sup> the bacteriology of colds and respiratory catarrhs has now been more or less exactly determined. The following list of causative organisms is probably nearly a complete one: The *Bacillus influ-*

<sup>1</sup> Local Government Board Reports, 1894-95.

<sup>2</sup> *British Medical Journal*, August 26, 1905.

<sup>3</sup> *Bull. Soc. Méd. Hôp. de Paris*, March 2 and 16, 1905.

<sup>4</sup> 'Catarrhal Fevers.'

<sup>5</sup> *British Medical Journal*, May 5, 1906, and November 6, 1909.

<sup>6</sup> *Lancet*, November 28, December 5, 1908; February 13, 1909, etc.



*enzæ*; the bacillus of Friedländer and its allies, including, possibly, the *B. proteus*; the *B. septus* (or *Coryzæ segmentosus*); the *Micrococcus catarrhalis*; *M. paratetragenus*; pneumococcus; and possibly the *Streptococcus salivarius* and the staphylococci. These may either be concerned singly or in conjunction one with another; some of them appear to be always with us, others are almost entirely epidemic in character; whilst others, though always with us, yet assume the rôle of catarrh-producers merely from time to time; any attempt, therefore, to express in percentage figures the relative frequency with which the various organisms are found were useless. At the same time each produces more or less its own type of cold, affects more or less a definite region of the respiratory tract, and, if unchecked, leads to complications more or less characteristic of itself alone. For fuller information on these points the reader is referred to the papers above mentioned by Benham and by the author. Careful consideration of the mode of onset, history of attack, and symptoms, will enable a provisional diagnosis of the causative organism or organisms to be made. This must then be confirmed or amended by microscopical examination of appropriate smears of the secretion stained by Gram's method, and counterstained for one minute with an aqueous solution of 1 per cent. neutral red, and by cultures prepared upon blood-agar plates by a series of strokes. On no account should the direct examination of smears be omitted, for certain organisms, such as the *Bacillus influenzae*, may fail to grow altogether, while less virile ones may be overgrown by those of more active growth. Any organisms appearing on the plate after twenty-four hours' incubation, which are not included in the above list, may be safely disregarded. It must also

be remembered that choice may not have been made of an appropriate piece of sputum or nasal discharge, or a swab may not have been taken from the proper locality. If, therefore, the bacteriological findings fail to correspond with the provisional diagnosis of the organism, fresh examination of the secretion should be made. This is a much-needed warning, as I once found out to a patient's cost. For the preceding year practically complete immunity had been secured; he then came to me with a hard, dry cough of three to four days' duration. The *Micrococcus paratetrigenus* was at the time causing many such colds, and was duly found. A dose of the corresponding vaccine failed materially to affect the cough. Three days later a fresh examination was made; a few *M. paratetrigenus* and *M. catarrhalis* were present both in smears and cultures, but the clinical picture did not altogether accord with this bacteriology. Unfortunately, a few days elapsed before another examination could be made. By this time double pneumonia supervened, and the patient nearly lost his life. When I failed at the second attempt to find the pneumococci suspected to be present, I should have insisted on making daily examinations till they were found or recovery had come about. Of course, it is not always possible to make these repeated examinations, and the trouble is considerable; whilst, on the other hand, there is danger in holding too strongly to preconceived ideas. Especially in the case of the aged and infirm, too great care can hardly be taken to establish a correct diagnosis of the infecting micro-organism, for by so doing many a case of pneumonia, arthritis, endocarditis, middle-ear disease, or meningitis, would be easily prevented. The careless and inaccurate diagnosis, 'Influenza,' has cost thousands of lives, and doubtless will

cost thousands more, until the profession at large awake to the dangers of the 'common cold,' and of the pneumococcal variety in particular, and realize the importance of making a precise bacteriological diagnosis.

Pneumococcal affections of the respiratory tract assume so many forms, and are so especially dangerous, that I make no apology for emphasizing the following points : In an acute attack the infection usually begins in the larynx or upper part of the trachea, but may begin in the nasopharynx ; the cough is paroxysmal and very distressing, and results in the expulsion of a tiny blob of very tenacious colourless mucus. The infection spreads simultaneously upwards and downwards, the mucus becomes much more copious, yellow, purulent, and less tenacious, but the distressing cough continues. Pulse and temperature may be little affected. The infection may stop at the bifurcation of the trachea, or it may spread into the bronchi and bronchioles and produce an acute bronchial catarrh. Herein lies the chief danger : after a longer or shorter interval the patient may apparently recover completely without vaccine treatment ; but if the bronchial secretion be examined, pneumococci will probably be found in abundance. Here they may lurk for weeks or months until climatic conditions or careless exposure to damp or draughts determines the lighting up of the dormant infection. The result of this may be bronchitis of the larger tubes, capillary bronchitis, pneumonia, or pleurisy. I have seen each of these sequelæ in those treated upon conventional lines, and if I can help it never allow a sufferer from a pneumococcal cold to dispense with vaccine treatment, this being continued if possible till careful examinations of the sputum no longer show the presence of the pneumococci. Such completely

satisfactory results are hereby obtained that neglect of a medical man to urge the necessity of vaccine treatment in a case of pneumococcal cold, especially in an aged subject, is little short of criminal.

In Table XI. an attempt is made to set out the rôle played by each of the catarrhal organisms, the particular part or parts of the respiratory tract which it most affects, and the commoner complications produced.

#### A. THE VACCINE TREATMENT OF ACUTE CATARRHS.

This may be initiated at any stage of the attack—the earlier the better. From the symptoms a provisional diagnosis of the organism may be made, to be confirmed, as stated before, by stained smears of the secretion and cultures upon blood-agar. If the smears confirm the diagnosis, a small dose of stock polyvalent vaccine may be given immediately. For the pneumococcus or *Bacillus influenzae* 25,000,000 will suffice ; for the others, 50,000,000 may be safely employed. The best site for injection is 1 to 2 inches below the centre of the clavicle ; the best time, between 7 and 10 p.m. A dose of Dover's powder or of the following mixture<sup>1</sup> should be given at the same time, the patient put warmly to bed in a well-ventilated room, and kept there, if possible, till convalescent ; or, if this be impossible, for twelve hours, at the expiration of which time the stage of increased resistance should have begun.

If the infection appear to be a mixed one, or if there

<sup>1</sup> R	Vin. ipecac.	..	..	..	..	℥v.
	Tinct. opii	..	..	..	..	℥x.
	Sp. ætheris nitrosi	..	..	..	..	ʒi.
	Liq. ammon. acet.	..	..	..	..	ʒiii.
	Aq. camphoris	..	..	..	..	ad ʒi.ss.

M. et f. haustus.

be any doubt as to its exact nature, it is better practice to await the results of culture and the preparation of the autogenous vaccine. In either case this latter should be used for the second inoculation, which may be given three to five days after the first, and of double the size.

The local results may be very similar to those due to an injection of antityphoid vaccine—viz., pain, redness, and some swelling—though occasionally these may all be absent.

The general effects are milder, but occasionally the patient passes a restless night, a cough is worse, there may be slight pyrexia (to 101° F.), headache, and malaise. Within twelve hours distinct improvement should have begun, and may be perceptible from hour to hour. Occasionally, however, this may be delayed for twenty-four hours. The most persistent symptom is, frequently, the cough.

The advantages of treating an acute attack of catarrh upon these lines are as follows :

1. Convalescence is speedier—sometimes almost complete in twenty-four to thirty-six hours.

2. The stage of thick mucous secretion is either practically eliminated or rendered much shorter and less distressing.

3. The use of expectorant drugs, which so often upset the digestion, may frequently be dispensed with.

4. The risk of complications is very considerably lessened. Indeed, I venture to say that, if this treatment were universally adopted, we should almost cease to hear of deaths due to pneumonia following upon so-called influenza, which in most cases is a pneumococcal cold pure and simple. Heart failure, arthritis, neuritis, and the other complications set out in Table XI., would be equally rare.

TABLE XI.

Organisms.	Acute Manifestations.	Chronic Manifestations.	Complications.
<i>Bacillus influenzae</i> ..	Nasal and bronchial catarrh.	Nasal catarrh, especially when complicated by empyema of the antrum of Highmore, frontal or ethmoidal sinuses.	Pneumonia (rare unless associated with the pneumococcus), septicaemia, endocarditis, meningitis, otitis media.
<i>Bacillus of Friedländer and its allies</i>	Nasal catarrh, ulcerative pharyngitis and tonsillitis.	Nasal catarrh.	Otitis media, pneumonia (rarely), abscesses (rarely).
<i>Bacillus septus</i> ..	Nasal catarrh, non-ulcerative pharyngitis.	None.	None.
<i>Micrococcus catarrhalis</i> ..	Nasal, tracheal, and bronchial catarrh, pharyngitis and laryngitis, bronchitis.	Catarrh of the whole respiratory tract and its accessory sinuses, Eustachian tube and middle ear, pyorrhœa alveolaris.	Otitis media, rarely meningitis, both spinal and cerebral.
<i>Micrococcus paratetragenus</i>	Nasal, tracheal, and bronchial catarrh.	Bronchial catarrh.	Very rare.
<i>Pneumococcus</i> ..	Nasal and tracheal catarrh, bronchitis, without any pneumonic symptoms, acute ulcerative pharyngitis, asthma.	Bronchitis, asthma.	Septicaemia, pneumonia (acute and chronic), empyema, arthritis, meningitis (spinal and cerebral), endocarditis, muscular abscesses.
<i>Streptococcus salivarius</i> ..	Bronchitis, asthma, tonsillitis.	Bronchitis, asthma, pyorrhœa alveolaris.	Septicaemia, endocarditis.



As mentioned above, the second dose may be given three to five days after the first. My usual practice now is to advise the administration, to those who are very prone to colds, of an immunizing dose of 125,000,000 to 250,000,000 of my combined vaccine for colds,<sup>1</sup> about ten days after the second. This dose may be repeated every three to four months.

## B. THE VACCINE THERAPY OF CHRONIC CATARRHS.

In dealing with chronic catarrhs, three points especially need keeping in mind: First, that chronic catarrh is usually associated with pyorrhœa alveolaris or follicular tonsillitis, and that the same organism may be responsible for both conditions. If this be the case, the futility of endeavouring to treat the catarrh successfully by means of a vaccine is obvious until the pyorrhœa or tonsillitis have been adequately dealt with by the dentist or surgeon. No vaccine will succeed in sterilizing a deep pyorrhœic pocket, or, if it should, the result will be but temporary. This also holds in the case of the tonsil. So convinced am I upon this point that I now absolutely refuse to treat a chronic catarrh if the patient refuses prior treatment of the stagnant pockets and crypts. Surgical attention to infected antra or sinuses is equally advisable. Second, some organisms exercise a restraining influence upon others, whilst others only prosper symbiotically. The bacillus of Friedländer inhibits the growth of other organisms, so that an infection may appear to be due to this organism alone. Its elimination by a course of injections may, however, be succeeded by an outburst of infection due, say, to the *Micrococcus*

<sup>1</sup> Obtainable from W. H. Martindale, 10, New Cavendish Street, W.



*catarrhalis*, which was previously held in restraint by the bacillus of Friedländer. Two or three doses of the new appropriate vaccine will in all probability complete the cure. Upon the other hand, it is rare to find the *Bacillus influenzae* alone causing pathological conditions of the respiratory tract. Rarely it does, especially when the accessory air-spaces are affected, but in these instances it often refuses to grow in artificial cultures. Much more commonly symbiosis exists with the pneumococcus, the *Micrococcus catarrhalis*, or the staphylococci. In this connection a very interesting fact may be mentioned: If in these latter cases a series of stroke cultures be made from a loopful of the secretion upon the surface of a blood-agar plate, it will be invariably noticed that the growth of the influenza bacillus is much more luxuriant in the neighbourhood of the colonies of pneumococcus, *Micrococcus catarrhalis*, or staphylococcus. I endeavoured to ascertain the cause of this, and found that if a few staphylococci were incorporated in the blood-agar mixture before pouring the plates, a similar added luxuriance of growth occurred in the influenza bacilli which were growing over a deep colony of the staphylococcus. As here no true symbiosis was present, the result was obviously due to some modification in the constitution of the blood-agar, possibly from the formation and diffusion of soluble exotoxin, although such is said not to be formed by the staphylococcus. The following experiment would tend to confirm this view: A loopful of pure culture of the staphylococcus was introduced into 10 c.c. of broth, and incubated for twenty-four hours. The broth growth was then heated at 60° C. for one hour, and proved to be sterile. To 10 c.c. of blood-agar mixture 2.5 c.c. of the sterilized broth culture was

added, and a plate prepared. Insemination of the surface of this with a loopful of *Bacillus influenzae*, which refused to be subcultured upon blood-agar without the addition of staphylococcic broth, resulted in profuse growth after eight hours' incubation at 37° C. By adoption of this method profuse growth of the *Bacillus influenzae*, which otherwise refuses to be cultured artificially, may frequently be secured. Into the pregnant considerations hereby suggested in connection with the genesis, nature, and significance of mixed infections, especially of the respiratory tract, I hope to go more fully at another time and in another place. In connection with the *B. influenzae*, it may also be mentioned that, so long as an infection remains strictly chronic, transmission to others by contact does not appear to occur; an exacerbation, however, usually results in those living in the same house also becoming attacked by the bacillus. During the past year I have had eleven such 'influenza-carriers' under treatment, coming from different parts of England, Ireland, and Scotland, and I am inclined to believe that it is to the lighting up by subtle alterations of climatic conditions of their chronic infection into an acute attack that the initiation of influenza epidemics is to be traced.

The third point which must be borne in mind to insure success regards the question of dosage. No upper limit for dosage can possibly be assigned; it depends entirely upon the patient's power of response. Thus, until recently I was afraid to exceed a dose of 500,000,000 of a vaccine of the bacillus of Friedländer. Encouraged by the effects of large doses of staphylococcic vaccine in obstinate cases of acne, I decided to try 1,000,000,000. In each of three cases the result was almost immediate

cure. The same holds true even to a greater extent in the case of chronic infections by the *Bacillus influenzae*. This is an organism against which the body immunizes itself extremely badly ; artificially an adequate response is also difficult to obtain. The vaccine apparently is but slightly toxic, and an initial dose of 100,000,000 can be employed with perfect safety. With a dose of 250,000,000 some pyrexia and malaise may ensue during the succeeding twenty-four hours, but soon passes off. A dosage of 500,000,000 will almost certainly, and of 1,000,000,000 very probably, have to be attained before freedom from the infection be secured. As I have said before, if one's prejudice is in favour of small doses, by all means begin with such ; but if the patient fail to improve markedly with these, cast aside prejudice and try larger and yet larger doses, for only this way is success secured.

The following are the highest dosages which, so far, I have been obliged to employ :

<i>Bacillus</i> of Friedländer ..	..	1,000,000,000
<i>Bacillus influenzae</i> ..	..	1,000,000,000
<i>Micrococcus catarrhalis</i> ..	..	1,000,000,000
<i>Micrococcus paratetragenus</i> ..	..	500,000,000
<i>Pneumococcus</i> .. ..	..	500,000,000
<i>Bacillus septus</i> .. ..	..	500,000,000

It is hardly necessary to mention that in cases where more than one catarrhal organism is present each of the appropriate vaccines will necessarily have to be employed.

Where infection is confined to the respiratory tract above the bifurcation of the trachea, even when accessory

sinuses are involved, uniform success should attend on vaccine treatment. A tendency to hypersecretion of mucus and to recurrent swelling of the nasal mucosa entirely independent of bacterial infection may, however, persist. When extension has occurred to the bronchioles and air-cells, the prognosis is less favourable, especially if emphysema or phthisis complicate the picture. Complete disappearance of the sputum is not often obtainable; diminution in amount and increased ease in its expulsion, lessened cough, and diminution in the number and severity of acute catarrhal attacks, can, however, certainly be secured. Treatment may have to be continued at seven to ten days' intervals for six to twelve months, and intermittently thereafter. In no badly emphysematous case have I ever been able to do more than improve the general condition, and diminish the quantity of sputum and the liability to acute attacks of bronchial catarrh.

#### BRONCHITIS AND ASTHMA.

If reference be made to Table XI., it will be seen that the organisms mentioned as being concerned in the production of bronchial catarrh or bronchitis are—arranged as far as possible in their order of importance—the pneumococcus, the *Micrococcus catarrhalis*, *Streptococcus salivarius*, *Micrococcus paratetragenus*, and *Bacillus influenzae*. These may either occur singly or in conjunction one with another. Single infection is much more common in acute bronchitis than in the chronic variety, and is much the more amenable to treatment. Among the irritants capable of setting up an acute attack of bronchial asthma, I have pointed out<sup>1</sup> that certain bacteria must be

<sup>1</sup> *Lancet*, September 11, 1909.

numbered. The commonest of these are, in order, the *Streptococcus salivarius*, pneumococcus, and *Micrococcus catarrhalis*. In a few cases, as the result of treatment by a vaccine of the apparently causative organism, the attacks of acute asthma have been diminished alike in number and severity, each injection being productive within twelve hours of a very short and mild, but none the less definite, asthmatic attack. At the same time it must be remembered that bacteria are only one of the agencies in any given case which are capable of setting up the asthmatic attack, and that, consequently, complete immunity can probably never be secured by vaccine therapy alone. In cases characterized by abundance of thin, frothy mucus this difficulty is also encountered: as the secretion is diminished in quantity it is increased in viscosity, and therefore in difficulty of expectoration. Due attention must be paid to all details, such as general health, climate, clothing, and food, in order that full benefit may be derived from a course of inoculations.

#### IMMUNITY AGAINST ACUTE CATARRHS.

The susceptibility of different individuals to the attacks of the various catarrhal organisms varies to a high degree. Some people are practically never free from an acute cold; others appear to enjoy practical immunity. This probably is due to some biochemical factor about which we, unfortunately, know nothing. The question therefore arises as to the possibility of protecting the susceptible against their microbic foes. The results of several years' experience lead me to conclude that such comes within the sphere of practicability. A close study of the clinical features presented by the attacks of several

preceding years will enable determination to be made of the particular organisms to which a given individual is especially susceptible, and it would appear to be a logical procedure to endeavour to secure immunity against these alone. In practice, however, I have found this to be not altogether satisfactory. In several instances where patients appeared to enjoy a natural immunity, say, against the pneumococcus, I accordingly omitted that particular organism from the immunizing mixture, with the annoying result that the next 'cold' epidemic which occurred was due to that microbe, and down went my patient under its attack. I therefore now think it best, when immunizing a patient against acute colds—which is done when they are free from any acute or chronic attack—to employ a mixed vaccine of all the known catarrhal organisms, or at least of each of the following: the *Bacillus influenzae*, *Bacillus septus*, *Micrococcus catarrhalis*, *Micrococcus paratetrigenus*, and pneumococcus. My combined vaccine for colds<sup>1</sup> now has this composition, and is put up in doses of 75,000,000 of each, 125,000,000, and 250,000,000, and is to be used as follows: The 75,000,000 dose is for use in the treatment of acute colds, when an autogenous vaccine cannot be prepared. Half this dose may be given at any stage of an acute attack, and may be followed in three to seven days by the full 75,000,000 dose. The 125,000,000 and 250,000,000 doses may be subsequently employed, but are more especially designed for securing protection against future attacks. The former dose is followed in ten days by the latter. If this procedure be repeated every three or four months, or, better still, whenever an epidemic of acute colds arises, practically complete immunity should be secured.

<sup>1</sup> Obtainable from W. H. Martindale, 10, New Cavendish Street, W.



## CHAPTER XI

### INFECTIONS BY THE BACILLUS TUBERCULOSIS GROUP

It must be admitted that progress in the vaccine treatment of tuberculous infections of the lung has been disappointingly slow. For this several factors have been responsible, among which may be mentioned :

1. The lingering hostility excited by the memory of ill results in the early days of tuberculin treatment, when this was purely in the experimental stage, and often fell into the hands of those who were little conversant with the powers of the drug of which they made use.

2. The aberration of mind which at the present day induces some to discard the lessons learnt from the therapeutic use of vaccines of other micro-organisms. No one will belittle the importance of employing an autogenous vaccine wherever possible in cases of infection, say, by the *Bacillus coli*, a term which now is known to be a generic one comprising many members ; yet few there are who admit the same advisability in dealing with the *Bacillus tuberculosis*, which is also a family of several individuals. In default of an autogenous vaccine, it is well recognized that a highly polyvalent vaccine is best suited for use in any given case, and I would maintain that precisely the same holds for the *Bacillus tuberculosis* as for any other micro-organism of variable character-



istics. Some, indeed, there are who have so far strayed from the rational path as to advocate the combat of an infection by the human strain with a vaccine prepared from the bovine strain, and *vice versa*. That cattle can be immunized against bovine infection by the aid of inoculations of the human strain—a procedure analogous with protection of the human being against variola by immunization with vaccinia—is no argument in favour of this procedure.

3. The belated recognition of the fact that many of the symptoms formerly ascribed to the tubercle bacillus are in reality due to secondary infection by other bacteria.

4. The incompleteness of our knowledge of the natural defensive mechanism of the body against infection and of the means whereby it is overcome, of the true nature of the tubercle bacillus, of its requirements for growth and of the toxins it forms. Of the peculiar properties of the tissues, which enable some to resist infection, while others fall an easy prey, we know nothing. This is almost certainly a biochemical problem, the constitutional defect being present in the embryo. Epiblastic and hypoblastic tissues seem more vulnerable than mesoblastic; and although in a given case more than one of these may be hypersusceptible, yet it would appear to be more or less the rule that, if infection begin in an epiblastic tissue, it is confined to epiblastic tissues, unless spread occur by contiguity. For instance, lupus, which is an infection of the epiblastic skin, seldom occurs along with tuberculosis of bones or joints, which are mesoblastic in origin; simultaneous infection of the glands, which are mesoblastic, and of the lungs, which are hypoblastic with the addition of some mesoblastic tissue, is relatively un-

common. The thorough investigation upon biochemical lines of young tissues, tuberculous and otherwise, would probably throw considerable light upon the causation of their vulnerability or resistive power respectively.

That the phenomenon noted on pp. 174, 175, in connection with the artificial culture of the *Bacillus influenzae* also obtains in relation to the growth outside the body of the tubercle bacillus, some incomplete experiments of mine appear to indicate (*vide* p. 224). Whether previous preparation of the living tissues by some other bacterium is an important adjunct to infection by the tubercle bacillus, future observation must determine.

#### THE DIFFERENT TYPES OF THE TUBERCLE BACILLUS.

Upon the identity or otherwise of the bacilli derived from typical cases of tuberculosis in human beings and cattle respectively, there has been much disputation. The balance of evidence appears to favour the view that there is no absolute distinction between them, and that intermediate forms capable of conversion into one or the other type may occur.

Inasmuch as the possibility must now be admitted of human beings becoming infected by the type peculiar to cattle, and of the latter developing pulmonary or other symptoms from infection by bacilli derived from and peculiar to the former, it has become necessary to devise tests whereby any given strain may be allocated with more or less certainty to its own particular group. The criteria employed are as follows : (1) cultural ; (2) inoculative ; (3) microscopic.

1. *The cultural characteristics* of typical members of the two groups are set out in the following table :

Human or Eugonic Type.	Bovine or Dysgonic Type.
<p>1. In <i>broth</i> rapid formation of thick, tough, wrinkled pellicle, which shows no tendency to sink. If the broth be slightly acidulated to begin with, this reaction never entirely disappears.</p> <p>2. On <i>glycerine agar</i> it usually forms a dense, warty, wrinkled layer.</p> <p>3. On <i>potato</i> there is rapid formation of a heaped-up richly pigmented growth.</p>	<p>1. In <i>broth</i> pellicle often slow in making its appearance ; generally very delicate, semi-translucent and speckled with a variable number of white spots ; occasionally it is opaque. It is very thin on the whole, and, with the exception of a few irregularly thickened areas, uniform. The acidity of the broth may be entirely neutralized and its reaction even become alkaline.</p> <p>2. On <i>glycerine agar</i> a thin grey haze on the surface is generally all that is to be seen at the end of five or six weeks.</p> <p>3. On <i>potato</i> at the end of five or six weeks the growth does not consist of more than a few grey colonies or streaks.</p>

Growths are, however, obtained from time to time which fail to conform with either of these types, and with these the second method of differentiation is especially necessary.

2. *Inoculation Method*.—This depends upon the marked difference in virulence towards cattle of the human and bovine types respectively. The assertion has been made by many eminent authorities that the human type is incapable of producing typical lesions in cattle. This is possibly not strictly accurate, but is sufficiently so for practical purposes ; and if, conformably with its cultural reactions, a given strain does or does not produce the typical lesions when injected into young calves, the

deduction may be made that the strain is on the one hand bovine, on the other human.

3. *Microscopical Appearances*.—Spengler,<sup>1</sup> by the use of special staining methods, states that the following differences may be made out : (1) That the bovine bacillus is the much larger and thicker, owing to the possession of a thick, sharply defined envelope composed of wax of low

TABLE XII.<sup>2</sup>

Investigated by—	Total Number of Cases in- vestigated.	Number of Cases in which Bovine Bacilli found.	Number of Cases in which Human Bacilli found.
British Commission ..	60	14	46
German Commission ..	138	22	116
	(of these 84 were children)	(of these 21 were children)	(of these 1 was a child)
Lewis .. .. .	15	9	6
Duval .. .. .	4	1	3
Ravenel .. .. .	2	1	1
Fife and Ravenel ..	1	1	0
Theobald Smith ..	12	1	11
Lartigau .. .. .	19	1	18
Vagades .. .. .	38	1	37
Fibiger and Jensen ..	12	7	5
Westenhoeffer .. ..	1	1	0
Ebers .. .. .	5	2	3
De Schweinitz, Dorset, and Shiroeder ..	9	2	7
Totals .. .. .	306	63 or 20 per cent.	243 or 80 per cent.

<sup>1</sup> *Wien. Med. Woch.*, 1902, No. 14 ; *Zeitschrift f. Hyg. u. Infect.*, Bd. xlix., 1905, etc.

<sup>2</sup> Moss, *Johns Hopkins Hosp. Bull.*, February, 1909, p. 48.

melting-point ; (2) that bovine bacilli may contain spores within them, while the human do not.

The author has examined several hundred preparations of sputum by Spengler's method, and cannot entirely confirm his bacteriological results. This may, of course, be due to errors of technique, but every effort was made to eliminate these.

By means, then, of studies of the morphology, staining reaction, cultural and biological characteristics, and pathogenicity, the bacilli derived from various sources of infection in human beings have been subjected to minute scrutiny. The collective results appear in Table XII. (p. 184).

Considering some of these in more detail, we find that Cobbett, working with the British Commission, obtained the results set out below :

TABLE XIII.

Nature of Case.	Number of Cases.	Nature of Strain of Bacillus isolated.			
		Bovine.	Human.	Irregular.	Human— Bovine on Passage.
Phthisis with tubercular sputum ..	4	1	2	—	1
Primary pulmonary phthisis .. ..	10	—	10	—	—
General tuberculosis	1	—	1	—	—
Tuberculous bronchial glands ..	4	—	2	—	2
Cervical glands ..	9	3	6	—	—
Primary abdominal glands .. ..	19	10	8	1	—
Joint .. ..	10	—	9	—	1
Testicle and kidney	2	—	2	—	—
Lupus .. ..	1	—	—	1	—
Total ..	60	14	40	2	4

Another observer gives the following results in eight cases :

TABLE XIV.

Nature of Case.	Age in Years.	Bovine Type.	Human Type.
Cervical adenitis .. ..	5	+	
Tuberculous tonsil .. ..	5	+	
Tuberculous tonsil and glands .. ..	2	+	
Meningitis and glands .. ..	3	+	
Genito-urinary .. ..	30		+
Miliary tuberculosis .. ..	21		+
Phthisis .. ..			+
Phthisis, peritonitis, and enteritis ..	61		+

The results obtained in children by the German Commission are set out in the following table :

TABLE XV.

Source of Material.	Number of Cases.	Type of Bacilli isolated.	
		Human.	Bovine.
Lungs, meninges ..	18	18	0
Abdominal glands ..	20	7	13
General miliary tuberculosis .. ..	1	—	1
Cervical glands .. ..	16	10	6
Bones and joints .. ..	27	26	1
Axillary glands .. ..	1	1	0
Skin (lupus) .. ..	1	1	—

Of the above, two showed double infection by the human and bovine types.

From these results it may be inferred that 50 per cent. of cases of tuberculous adenitis in children are bovine in origin, but that, despite this, practically all cases of

pulmonary phthisis in children are caused by bacilli of the human type. In the material from the fifty-four cases of tuberculosis in adults (including four cases of adenitis), the human type was found in every instance. In one case both types were present.

The disparity in the results set out in Table XII., obtained by different observers, is easy of explanation. Thus, Lewis, who in fifteen cases found the bovine type in nine, examined only cervical glands, while Vagades, who in twenty-eight cases found the bovine type once only, obtained his cultures from sputum or the lungs at autopsy in all but one instance.

Cases of mixed infection by both types are also on record, and are probably of much commoner occurrence than would appear from the statistics, owing to the fact that, if both be present in the pathological specimen, cultures will nevertheless tend to display the characteristics of the less virulent but much more luxuriant human type. On the other hand, if passage through animals resistant to one or the other type be employed for isolation purposes, then that type may fail to survive.

That to some fallacy is due the apparent inability of the bovine type to cause pulmonary lesions in the adult, I cannot but feel. If 50 per cent. of cases of glandular tuberculosis in infants are caused by the bovine type, while no cases of pulmonary phthisis, and but few of tuberculous adenitis, in the adult are due to the same cause, then we are faced by the following alternatives :

(a) Glandular tuberculosis caused by the bovine type in the infant never leads to pulmonary phthisis in the adult.

(b) If it does, then the bovine type by long residence becomes transmuted into the human type.



(c) Glandular tuberculosis in the infant due to the bovine type practically always gets well ; only that caused by the human type persists.

(d) If glandular tuberculosis due to the bovine type persists till adolescence, then the type becomes transmuted.

Proof has not been afforded of any of these necessary corollaries to the theorem, and I feel with Spengler that cases of pure bovine and of mixed human and bovine pulmonary infection are much more common than is supposed to be the case. Further consideration of this point is deferred till later.

#### TOXINS FORMED BY THE BACILLUS TUBERCULOSIS.

These are very imperfectly understood. When the bacilli are grown in alkaline glycerine broth, toxic products are found in the culture fluid, but whether these are exotoxins or endotoxins liberated by autolysis of the bacilli is not known. The bodies of the bacilli are toxic even when dead. The fatty acid which confers on the bacillus its peculiar staining reaction is said to be the cause of the resultant chronic inflammatory and caseous changes in the tissues. Another substance is said to cause rise of temperature, but it must be remembered that tuberculosis may be an apyrexial disease throughout, and that mixed infections play a predominant part in the causation of pyrexia. Certain volatile constituents are said to have a convulsant action. The main toxic effect produced by the tubercle bacillus would appear to be a local one upon the tissues surrounding an infected focus, the symptoms of any general toxæmia being little in evidence.

## IMMUNITY AGAINST THE TUBERCLE BACILLUS.

As to the production of immunity against the tubercle bacillus we know practically nothing. One attack does not seem to confer any immunity against another, but, on the contrary, rather to predispose to it. That general constitutional immunity, due probably to biochemical causes, exists is highly probable, but local immunity appears to be a most important defensive mechanism.

Phagocytosis apparently is the most important agency in the destruction of the bacilli, and is carried out almost entirely by the giant and endothelial cells. Whether the polymorphonuclear leucocyte plays any part—except, perhaps, in cases where the bacilli have gained access to the general circulation—is very doubtful. Opsonin, partly of the thermostable variety, but chiefly thermolabile, can be demonstrated in the blood-serum; but whether it is necessary for sensitization of the bacilli prior to phagocytosis by the giant and endothelial cells has not been determined.

Wassermann and others have demonstrated the presence of antibodies in those who have been immunized with tuberculin, but their nature is not known. No bacteriolysin, bactericidin, or antitoxin, has been proved to exist. An agglutinin is often formed, but is sometimes absent. Although its importance has been disputed, my own clinical experience is that those cases where marked clumping of the bacteria is to be seen in the sputum almost certainly get well.

METHODS OF DIAGNOSIS OF TUBERCULOUS INFECTION,  
AND OF THE TYPE OF THE ORGANISM CONCERNED.

(A) *By Clinical Signs.*—These will be found fully described in any such work as Pottenger's 'Pulmonary Tuberculosis.'

(B) *Special Methods not Dependent upon Determination of the Index.*

1. *The Old Tuberculin Test.*—If a healthy individual receive an injection even so large as 0.01 c.c. of old tuberculin (Koch), no symptoms beyond slight local tenderness will be exhibited. The case is very different with a person afflicted with tuberculosis, especially if in an early stage. If the dose of tuberculin be extremely small, no effect may be noted; if a larger, a local hyperæmia of the infected area; if still larger, a congestion; while if larger still, a constitutional disturbance of varying degrees of severity will result. If the infected areas be visible, as in the larynx or pharynx, the hyperæmia and congestion can be readily detected. In the lung there is an increase in the symptoms, confined to the area of infection; the auscultatory signs are magnified, and resemble a catarrhal condition of greater degree. Fine râles may appear where none were to be found previously, or their number may increase. Careful charting of the signs before and during the reaction is therefore necessary.

It is possible to have this local reaction without any general one. If the latter be present, a few hours after the administration of a small dose of tuberculin the patient begins to feel a little nervous or tired, and perhaps has a heavy feeling about the limbs. With this there may be a slight rise of temperature of a fraction of

a degree or a slightly accelerated pulse. With a larger dose the tired feeling and heaviness of the limbs becomes a true ache, which extends to the back and head, and the feeling is that of an oncoming cold. With this the temperature usually rises one or two degrees, and the patient may develop a cough where none was present before. If the dose be still larger, the patient may have a rigor, and nausea and vomiting occur.

The more experienced the physician, the less the amount of general reaction that he requires to establish a diagnosis, and an endeavour is made so to adjust the dose that a rise, at all events, of not more than  $1^{\circ}$  F. shall occur in the temperature. If this be already above  $100^{\circ}$  F., the use of the test is contra-indicated—at all events, until rest and other appropriate means have reduced the temperature to the region of the normal.

Inasmuch as the reaction usually shows itself in from eight to twenty hours, the dose of tuberculin is best given at eight or nine o'clock at night, the temperature being then taken at six o'clock next morning and at two-hourly intervals. Examination of the chest for the local reaction should begin at the same time, and be repeated at three to four hourly intervals until the presence or absence of local reaction is established. For the purpose of the test Koch's old tuberculin is usually employed. Some people being very sensitive to it, it is best to begin with a dose of only 0.0001 c.c., to which only very exceptionally is any response made. Should no reaction occur, the dose is increased; 0.001, 0.003, 0.005, 0.007, 0.01, 0.1 c.c. being used in successive intervals of one or two days until a positive result is secured, as is usually the case with the second or third of these doses in tuberculous cases. A negative result with the last of

these doses is considered to be final, so far as infection by the human type is concerned. If tuberculosis be strongly suspected, repetition with tuberculin of bovine origin is obviously necessary to exclude infection. In view of the importance of determining whether one or both types of the bacillus are at work, advantage may be taken of Spengler's observation that a patient infected solely or mainly with bovine bacilli is most sensitive to the toxins of the bovine bacillus, while one infected solely or principally with human is most sensitive to the toxins of the human bacillus. Upon the other hand, the tuberculin of one variety appears to act as an antitoxin in infections by the other variety. The test is therefore carried out as follows : The patient is put to bed till the temperature chart no longer shows abnormal fluctuation, and tuberculin of human origin is given diagnostically till a definite temperature reaction is obtained, or, failing that, till the maximum dose has been given. In the former event, the administration of the same or a slightly larger dose of tuberculin of bovine origin should almost at once re-establish the temperature either to its former level or to a lower one. In the latter case it should result in a rise of temperature, which will be correspondingly depressed by another similar dose of tuberculin of human origin. It must be remembered that some cases of advanced phthisis will not respond to the test. It has also been stated that certain cases of syphilis have given a positive reaction, but against this it must be borne in mind that sufficient proof that these cases were not also infected somewhere by the tubercle bacillus has not been always forthcoming.

*Contra-indications* to use of the test are : (1) If the temperature rises above  $98.6^{\circ}$  in the axilla, or  $99^{\circ}$  F. in the

mouth; (2) if definite signs of tuberculosis be present, if tubercle bacilli be present in the sputum, or if there has been a recent attack of hæmoptysis; (3) if there be grave renal or cardiac trouble; (4) if the patient be subject to epileptic fits.

2. *Von Pirquet's Cutaneous Reaction*.<sup>1</sup>—Von Pirquet employed a solution consisting of Koch's old tuberculin 1 part, 5 per cent. carbolic acid 1 part, normal salt solution 2 parts, and showed that by its means a reaction could be obtained in persons infected by the tubercle bacillus, which is but rarely obtainable with healthy individuals. The skin of the upper arm is cleansed with ether. Two drops of the above solution are then placed on the skin about 2 inches apart, and the skin slightly abraded by the aid of a lancet, which is then disinfected, and a third abrasion made between the other two, or upon the other arm, to serve as a control.

After forty-eight hours some redness and œdema result, and a papule resembling that of vaccinia soon appears at the two infected areas, but not at the control one. Within a week the pustule dries up, and the reaction subsides. Occasionally the reaction is delayed for two or three days.

The advantage of this test is that, as there is no temperature reaction, it can be employed in pyrexial cases and in children without fear of constitutional disturbance. It has been found especially trustworthy in the first three years of life; it is not quite so reliable after that up to ten or twelve, and still less so afterwards, especially in the very cachectic. If no reaction occur, it is obviously necessary to repeat the observation with tuberculin of bovine origin before arriving at a negative conclusion.

<sup>1</sup> *Deut. Med. Woch.*, May 23 and 30, 1907.



Morro's percutaneous method is a modification of this, equal portions of old tuberculin and lanolin being mixed up together and applied to a small area of the skin of the abdomen till absorption has occurred.

3. *The Ophthalmic-Reaction of Calmette and Wolff Eisner.*—These two investigators simultaneously discovered that a diagnosis of tuberculosis can be established in a very large percentage of cases in the following way :

By means of 95 per cent. alcohol a precipitate is obtained from Koch's old tuberculin. This is washed and dried, and made up in a solution of normal salt solution to a strength of 0.5 per cent. One or two drops of this solution are introduced into the eye of the person supposed to be tuberculous, near the inner canthus, and the eye kept open for a few seconds. A positive reaction appears in from eight to twenty-four hours, and begins with lachrymation, going on to reddening of the conjunctiva, and in severe cases to fibrinous exudation. The amount of reaction is no evidence of the severity of the disease, and varies from slight injection of the caruncle—which can only be detected by comparison with the untreated eye—to all the signs of a severe purulent conjunctivitis with œdema of the lids. The reaction usually passes off in three to four days, but may last for a week, or even longer. As a rule, little discomfort is caused, but occasionally there may be sufficient pain to require treatment. In cases free from tuberculous infection no reaction soever is obtained.

From a summary of all the published observations, it would appear that 95 per cent. of all cases suffering from active tuberculous infection give a positive reaction. Moribund cases and a few of miliary tuberculosis refuse to respond; while in cases supposed to be quiescent either



a positive or a negative result may be obtained. The possibility of failure being due to the fact that infection may be of bovine origin should not be lost sight of. The rapidity and intensity of the reaction are said to be in direct ratio to the vigour with which the body is defending itself against the infective process ; this may account for its late appearance, or even failure, in very long standing caseous and cachectic cases, and in very extensive or very active lesions, such as acute miliary tuberculosis, meningitis, or peritonitis. At the same time its prognostic value is said to be slight, as the production of antibodies, which governs the intensity of the reaction, may bear no relationship to the gravity of the disease. Nevertheless, when a negative reaction is secured where previously it was positive, and coincident with this there has been disappearance of clinical signs, anatomical cure of the tubercles may be assumed to have occurred.

Any affection soever of the eye already present is an absolute contra-indication to the employment of this test. Several accidents, such as corneal ulceration and iritis, have been reported to have resulted from its use, and have led to the employment of a solution of 1 in 200 instead of one of double this strength, as was first advocated. It is, perhaps, advisable to use a still weaker solution—say 1 in 500—in the first place, and only if this prove negative, one of a strength of 1 in 200 two or three days later. The accidents which undoubtedly did occasionally follow the use of the 1 per cent. solution have tended to bring this test into a certain amount of discredit, but in careful hands these may well be obviated in the future ; and this most valuable method of diagnosis in doubtful cases occupies a deservedly assured position.

#### 4. *The method of observing deviation of complement*

(Gengou-Bordet effect) must be mentioned, but the difficulties of carrying out the test are too great to enable it to be employed in practical diagnosis.

(C) *Special Methods Dependent upon Observations of the Opsonic Index.*—These have all been referred to previously, but are here recapitulated that attention may be drawn to a few important points.

1. Simple determination of the index, repeated if necessary.

2. Determination of the index before, and at various intervals after, the production of an auto-inoculation by movement, massage, Bier's congestion, etc.

3. In women comparative determinations before and during a menstrual period.

4. Determination of the index before injection of a small dose, say 0.00002 c.c. T.R., and one day, two days, and ten days after, with a view to the observation of the extent and duration of the resulting negative phase, which in healthy people will be very slight and of short duration—viz., amount to 0.1 or 0.2 and last one or two days. In the infected it will be greater and of longer duration—viz., 0.3 or 0.4—and may last even a week or a fortnight, or longer (*vide* p. 68).

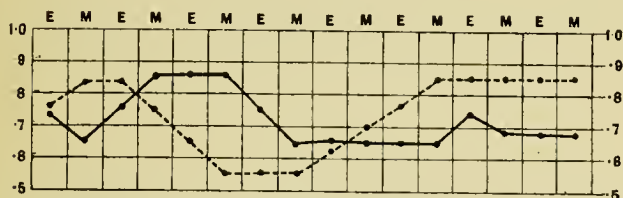
It must be noted that, if the patient be infected by bacilli of the bovine type, no response may be elicited by injection with T.R. of human origin, but will be by T.R. of bovine origin. This failure to respond to T.R. of human origin was noted by Stewart and Ritchie in about 10 per cent. of their cases,<sup>1</sup> and in eleven cases of Ritchie, referred to on p. 76.

It is, therefore, best in performing the test to use both T.R.'s and estimate the indices to both varieties of the bacillus.

<sup>1</sup> *Edinburgh Medical Journal*, May, 1907.

The index in pulmonary tuberculosis to the human and bovine types respectively, and the effect thereupon of injections of the two T.R.'s, has been investigated in a number of cases by Williamson, who has kindly supplied me with the following charts (Nos. XVI.-XX.) :

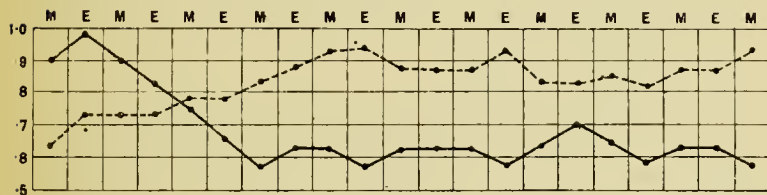
CHART XVI.



Index determined morning and evening, patient resting in bed. Dotted line=index to bovine type; continuous line=index to human type.

Chart No. XVI. is one typical of six cases. The sputum contained abundant tubercle bacilli, and the patient rested in bed during the estimations. It will be

CHART XVII.



Index determined morning and evening, patient in bed; heavy lung exercises. Patient gained 13 pounds in weight. Lines indicate as before.

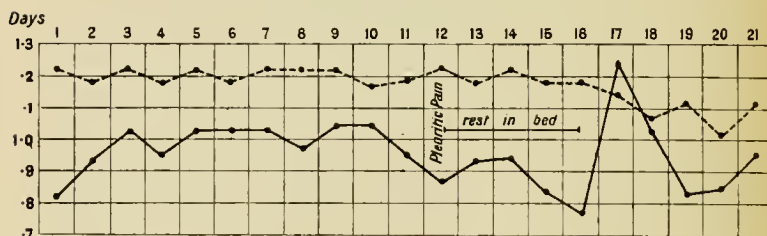
seen that the indices to the human and bovine types respectively moved in the same direction, but not synchronously.

Chart No. XVII. exhibits similar features. Tubercle bacilli were numerous, and, according to Spengler's methods of staining, both types appeared to be present.

It will be observed that, although the index to the bovine type rose from 0.6 to 0.94, that to the human fell from 1.0 to 0.6; despite this, the patient did very well, and gained 13 pounds in weight. The more important of the two infections would therefore appear to have been the bovine one.

That the indices to the two types do not always move more or less together is seen in Chart No. XVIII. The onset of a slight pleuritic attack raised the index to the

CHART XVIII.



Daily determinations of index. Few tubercle bacilli in sputum. Temperature normal morning and evening upon graduated exercises. Upon the twelfth day developed a pleuritic pain, and was accordingly in bed till the sixteenth day. High index to the human strain upon the seventeenth day confirmed by a duplicate estimation.

human type from 0.8 to 1.2, without influencing that to the bovine type to anything but a slight extent; the infection in this case was obviously one by the human type alone.

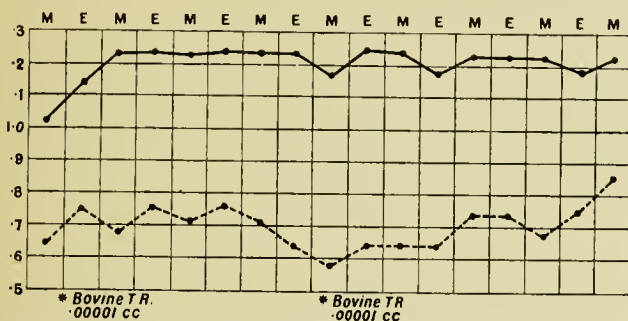
The effect of injection of a human T.R. upon the index to the human bacillus has been fully described in the text; the effect upon the index to the bovine bacillus requires further elucidation.

From Chart No. XIX. it will be seen that an injection of 0.00001 c.c. of bovine T.R. had no effect upon the index to the human type in a case of advanced phthisis, whereas

it produced a definite but slight rise in the index to the bovine type.

Chart No. XX. shows very well the effects of injection

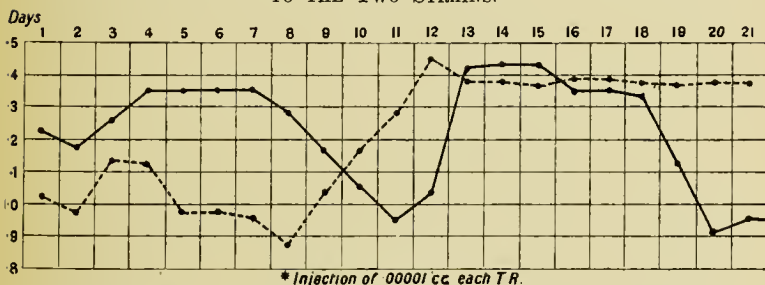
CHART XIX.



*Case of Very Advanced Phthisis, with Moderate Inverse Pyrexia.*—Owing, perhaps, to advanced state of disease, immunizing response to injections poor. The indices point to bovine nature of infection.

CHART XX.

TO SHOW EFFECT OF INJECTION OF MIXED T.R.'S UPON THE INDICES TO THE TWO STRAINS.



*Case of Early Phthisis.*—Upon the eighth day 0.00001 c.c. of each T.R. (human and bovine) were given. The effect upon the indices is seen in the chart. The temperature remained normal, the moist sounds were multiplied, and the sputum became slightly blood-stained.

of the mixed T.R.'s as advocated by the author. It will be seen that the indices to both types rose most satisfactorily. The dosage of the T.R.'s given was so small

that no effect was produced upon the temperature ; the moist sounds were, however, multiplied, and the character of the sputum altered for a day or two after the inoculation.

These charts are only typical of many, and the work is to be continued ; but, so far as it goes, it appears to justify the author's contention that the human T.R. raises the index to the human bacillus, and only slightly affects that to the bovine bacillus, and *vice versa* ; while an injection of the mixed T.R.'s causes elevation of the index to both.

5. The absorption method of Peel Ritchie (*vide* p. 75), with diluted and undiluted, heated and unheated, sera.

The sera of two of the eleven cases there referred to which failed to give the absorption reaction with tubercle bacilli of human origin were tested with bacilli of bovine origin, and gave the characteristic reaction. Upon testing eight cases of abdominal tuberculosis by this method, five were found to be due to infection by the bovine type, three by the human ; four cases of cervical adenitis all proved to be of bovine origin.

#### VARIETIES OF TUBERCULIN ; THEIR EFFECTS AND SUITABILITY FOR ANY GIVEN CASE.

The preparations of the tubercle bacillus employed in the attempted production of immunity are very numerous, the differences in many instances being too slight to be worthy of mention. Perhaps the best method of division is according as to whether use is made of the products which diffuse into a liquid culture medium, either as the result of the growth or the autolysis of the bacteria, or whether the whole or part of the bodies of the comminuted bacilli enter into the preparation.



Tuberculin was originally prepared by Koch by growing the bacilli from six to eight weeks in flasks containing slightly alkaline veal broth, to which peptone and glycerine had been added, a free supply of oxygen being maintained throughout. By filtration through sterile porcelain the bodies of the bacteria were removed, the resultant filtrate being then concentrated by heat to a tenth of its original bulk. It thus really consisted of a solution in glycerine of the soluble products of the bacterial growth. Analysis showed it to be a very complex substance, among other bodies alkaloids, albumoses, and extractives, being contained therein. To one albumose the local inflammatory reaction produced by its injection was attributed, to another albumose its curative effect, while to the extractives the resultant pyrexia was attributed. Similar analyses of the various other preparations do not appear to have been made.

In Table XVI. are set forth the commoner modifications, with their main characteristics.

The mere fact that so many varieties of tuberculin have been prepared—those comprised in Table XVI. being but a fraction of the total number—is in itself sufficient testimony of the fact that none has given complete satisfaction. In cases where toxæmic symptoms are but slight, a vaccine prepared from the triturated bodies of the bacilli and the endotoxins therein contained would appear to be sufficient. Such a preparation is the bacillary emulsion, or B.E. Where toxæmia is a prominent symptom, a preparation capable of stimulating anti-toxin formation is indicated. Such a one is Beraneck's tuberculin, or in lieu thereof the bacillary emulsion may be combined with Denys' tuberculin, or with old tuberculin (vacuum preparation). That careful differentiation



TABLE XVI.

A. Exotoxigenic media products	1. Old Tuberculin, Koch (T.O.A., or Tuberculinum Original Alt): A filtered broth culture concentrated by heat.	Productive of antitoxic immunity.
	2. T.O. (Tuberculinum Oberer, Koch and Von Ruch): Products yielded up to water by the comminuted bacilli.	
	3. Vacuum Tuberculin { Same as Old Tuberculin, save that no heat is employed	
	4. Bouillon Filtrate (Denys) { in concentration.	
B. Endotoxigenic cellular products	1. T.R. (i.e., Tuberculinum Rückstand): A heat-concentrated part-cellular product (2 milligrammes of the extract from 10 milligrammes of bacilli dissolved in 1 c.c. of aqueous glycerine).	Productive of antibacterial immunity.
	2. Vacuum T.R.: Same as 1, except that heat is not employed for concentration purposes.	
	3. B.E. (Bacillary Emulsion): A total cellular product, consisting of the comminuted bodies of 5 milligrammes of dried tubercle bacilli suspended in 1 c.c. of 50 per cent. aqueous glycerine.	
	4. Tuberal (Thamm): Part-cellular product, being a greyish-white albuminous extractive of the bacilli dissolved in 0.3 per cent. carbolic acid.	
C. Exotoxigenic medium product and endotoxigenic cellular product	Beraneck's Tuberculin, which is prepared without the aid of heat, and consists of a mixture of equal parts of the exotoxins diffused into alkaline veal broth, and of the endotoxin prepared by extraction of the bodies of the bacilli.	Possibly productive of both antitoxic and antibacterial immunity.

These may be prepared from bacilli either of the human or bovine variety. In the latter instance they are differentiated by prefixing the initial P (= *perlsucht*) before those of the corresponding product from the human strain. Thus, P.T.R. = *Perlsucht Tuberculinum Rückstand*, or *Bovine T.R.*

be made between toxæmic symptoms due to the tubercle bacillus and to secondary infection respectively is of the utmost importance in this connection.

Let it be granted that by means of the methods already indicated determination has been made as to the precise nature of the infection, whether due to the human or the bovine variety, or to both in conjunction, and choice made of the form of tuberculin that is to be employed : it remains now to consider from what variety of bacillus the tuberculin should have been prepared. I would maintain that the one lesson above all which experience of vaccine therapy has taught is the value of an autogenous vaccine, and what holds for other pathogenic organisms holds with no less force in the case of the tubercle bacillus. In default of the autogenous vaccine—and in this instance its preparation means both considerable expenditure and loss of time—experience has amply demonstrated the value of polyvalency. For the moment I care not whether the human and bovine types are essentially the same or essentially different. Let it be admitted merely that there are many varieties of the tubercle bacillus, and the advisability of polyvalency in tuberculin can hardly be questioned. If the use of human T.R. has been decided upon, then I would urge that a mixture of several T.R.'s from as many different sources is likely to have an additional value over one prepared from a single strain of tubercle bacillus.

To sum up, unless the tubercle bacillus differ in its mode of action and in its effects from all the other pathogenic micro-organisms with which vaccine therapy has dealt, the following deductions must hold :

1. That an autogenous vaccine must have the highest value.

2. That in default of the autogenous vaccine use should be made of a polyvalent one.

3. That this polyvalent vaccine should correspond in type to the infecting strain or strains.

To the last of these certain well-known authorities refuse assent, partly from theoretical, partly from experimental considerations. Spengler and Raw argue as follows : Romburg and Behring have shown that cattle, which are for practical purposes immune against the human strain, can be most completely immunized against infection by the bovine strain by inoculation with the human ; therefore, conversely, human beings can be best immunized against infection by the human type by means of tuberculin of bovine origin, and *vice versa*.

To this proposition the following objections are to be preferred : In the process of immunization, living human bacilli are finally employed. This is possible because they fail to produce generalized tuberculosis in bovines. Could living bovine bacilli be used for the purpose in equal numbers, the immunity produced would probably be even more complete. Again, it is hardly permissible to assume that what holds for bovines necessarily holds for human beings. The human type seldom produces generalized tuberculosis in bovines, but the bovine type does in humans.

Spengler has observed that human tuberculin appears to be the more toxic in infections by the human strain, and bovine tuberculin in infections by the bovine strain, which is an observation entirely in accord with what obtains generally in vaccine inoculations. He, however, carries the matter farther, and states that the rise of temperature produced in a case of infection by the human type by an inoculation with human tuberculin can

be controlled and neutralized by a succeeding dose of bovine tuberculin, and in this observation he is confirmed by Pottenger,<sup>1</sup> Bandelier and Roepke, and others. His deduction, therefore, is that bovine tuberculin either is antitoxic to, or produces an antitoxin to, the toxins elaborated by the human type bacillus, and *vice versa*. Of the truth of the observations there is little or no doubt; of the truth of the deduction laboratory proof is lacking, and it appears theoretically to be unsound. Nor is this all. Even granting that his conclusion is right, it does not necessarily prove the value of the procedure. What is required is the stimulation of bactericidal rather than of antitoxic substances, and as to whether this object is secured we know nothing.

Personally I regard the matter from quite a different aspect. My experience, as well as that of many other vaccine therapists, is that a moderate temperature reaction is a most desirable sequel to a therapeutic inoculation, and is taken to indicate adequate immunizing response. It is probably partly due to liberation of toxin from death of some of the bacilli at the infective focus, and is as a rule followed by a fall of temperature to a lower level than that at which it stood prior to inoculation. There is little need to mention that the rise should not be excessive—*i.e.*, over 1.5° F.—or overdosage is indicated; nor that occasionally a fall occurs, especially in systemic infections, without any precedent rise.

Precisely the same thing may be considered to occur in tuberculous infections, rise in temperature being partly indicative of death of the bacilli at the infected focus. If it be excessive, the dose of tuberculin was too large, more toxin being liberated than the body could adequately cope with,

<sup>1</sup> 'Pulmonary Tuberculosis,' p. 179, and figs. 28a and 28b.

a moderate rise succeeding by a fall to a yet lower level being indicative of adequate immunizing response. Should the rise of temperature be excessive or too prolonged, then it is most useful to be able to control it by means of an inoculation of the opposite kind of tuberculin. I may add that, although during the past three years I have treated with tuberculin the most diverse varieties of tuberculous lesions, I have very rarely indeed had a temperature response of over  $1^{\circ}$  to any inoculation. Whether this be due to the small dosage employed, or to the fact that in the majority of cases I have used a polyvalent tuberculin of both human and bovine strains, I am unable to say.

#### METHODS OF EMPLOYING THE VARIOUS TUBERCULINS IN PULMONARY PHTHISIS.

Assuming that the diagnosis of tuberculosis has been confirmed by one of the methods already mentioned, the variety of bacillus determined either by direct or indirect means, and the kind of tuberculin to be used decided upon, the important question remains as to how the administration of the drug is to be carried out. Several alternatives present themselves :

1. It may be done according to the approved Continental or intensive method, with or without the modifications recommended by Spengler and approved by Pottinger and others.

2. The routine control of the opsonic index may be utilized.

3. Or, finally, pulse, temperature, and clinical signs and symptoms, may be employed to control a scheme of dosage more or less in accordance with the experience of opsonic index methods.

Reliable statistics are lacking which would enable us to arrive at a definite decision as to which of the above methods consistently yields the best results. Personally I feel that some cases will do best under one method, others under another. Tuberculin is no definite specific for pulmonary tuberculosis ; it is only an adjuvant—admittedly a very valuable one—to other methods of treatment, and probably fails directly to influence the biochemical defect which permits infection to occur. In advanced cases, moreover, the secondary infections are more important in several ways than the primary infection by the tubercle bacillus, as will be mentioned later. So many factors may be at work in any given case of pulmonary phthisis that to apprise the results produced by a course of tuberculin is an extremely difficult matter. Success is gained by minute attention to details, by placing the patient under the best possible conditions, and by so keeping an open mind as to the influence of a course of specific treatment as to be prepared to modify it to suit the individual patient.

*A. Immunization under Guidance of Clinical Symptoms,  
as practised upon the Continent.*

Slight modifications of dosage are introduced by different authorities in accordance with their own clinical experience and to suit the individual patient ; but the following directions may be taken as indicating the procedure in the average case, whether the human or the bovine preparation be employed :

Old tuberculin (Koch)—either the T.O. or the T.O.A.—is now chiefly employed for diagnostic purposes. It is used therapeutically as follows :



For patients in the first and second stages, in whom prognosis is still good and who are free from fever, the initial dose is from 0·0001 c.c. to 0·001 c.c. This is gradually increased by equal amounts, and is given every third or fourth day, according to symptoms, until 0·005 c.c. has been given, when the increase may be by 0·002 c.c., until 0·01 c.c. is reached, when the increase may be more rapid. A feeling of nervousness, malaise, or aching, either with or without a slight rise of temperature, is to be taken as a sign of reaction. The dose should then not be repeated until this has disappeared, and the amount should not be increased until this amount, when injected, fails to produce these symptoms. If all goes well, the dose may be increased thus : 0·015, 0·02, 0·03, 0·04, 0·06, 0·08 c.c., given at properly spaced intervals ; but this should not be attempted by one who does not understand the remedy well. Sometimes patients are very sensitive to tuberculin, and the dose can then only be increased very cautiously, it taking even one or two months to attain a dosage of 0·001 c.c.

In cases in the third stage the initial dose is 0·00001 c.c., and it will often be found impossible to raise the dose beyond 0·001 c.c. to 0·01 c.c. in such cases without producing a reaction.

In almost all cases the maximum dose should be repeated several times at increasing intervals of weeks after the patient is apparently cured. Bandelier and Roepke<sup>1</sup> state that, as experience increases, the contra-indications to this line of treatment grow fewer and fewer, and that they have treated with success, or, at any rate, marked improvement, advanced cases with hectic fever, purulent expectoration, cavities in the lungs, anæmia, and emaciation.

<sup>1</sup> *Lehr. der Spezif. Diag. u. Ther. der Tuber.*, Würzburg, 1908.



Koch also recommends the intravenous use of this preparation in very small doses in cases undergoing treatment with T.R., in whom toxæmic symptoms continue prominent. Spengler finds the bovine form the less toxic, and at the same time the more active in stimulating the machinery of immunization, as measured by the power to increase the specific agglutinins of the blood. The procedure is exactly the same as with the human variety. Spengler, however, considers the least feeling of warmth at the site of injection as sufficient to negative a fresh injection until this sign of reaction has subsided. He believes that only patients *in extremis*, or with very acute pulmonary phthisis, are beyond its reach. Bandelier and Roepke (*loc. cit.*) report satisfactory results in 178 cases. Fever and the presence of mixed infection are not contra-indications to its use.

Tuberculin (Denys) is, like the above, a toxic medium product. It is a filtrate made from the bouillon in which the bacilli have grown, and is prepared without being subject to heat, and is not concentrated like Koch's old tuberculin.

The pure tuberculin is taken, and seven successive dilutions made, each of a tenth of the strength of the preceding, so that the final, or No. 7 dilution, contains 0·0000001 c.c. of the original tuberculin in each c.c. This is the initial dose in febrile cases ; in afebrile cases 1 c.c. of the No. 4 dilution = 0·0001 c.c. of the original tuberculin, is employed.

In either case the increase in dosage is by 0·1 c.c. at intervals of three to four days for the smaller doses ; as the higher doses are reached, five or six days must elapse, and for the last three or four doses, when the pure tuberculin is being used, intervals of a week or ten days must

be allowed. The final dose is 1 c.c. of pure tuberculin. If no intolerance be evidenced, the course of treatment will extend over six months, but when reactions occur it may need a year. In the event of a reaction it is necessary to await its disappearance, and begin again with half the dose that produced it. They occur the more readily at doses between 0·0001 and 0·001 c.c. Denys refers to 2,000 cases as having been treated successfully by him according to this procedure.

T.R. (human) and P.T.R. (bovine) are endotoxic cellular products prepared from the human and bovine types respectively, and contain 2 milligrammes of bacillary extract, corresponding to 10 milligrammes of bacilli per c.c. They are probably the mildest of all the forms of tuberculin, and are productive of antibacterial, and only to a slight extent of antitoxic, immunity. They are therefore especially suitable for the treatment of patients who are very sensitive to old tuberculin, and may be used to pave the way for the use of the latter. The initial dose is 0·0001 to 0·0002 c.c., and may be given every other day at first. It can usually be doubled at each dose, until 0·01 c.c. of the original has been given. After this the injections should be farther apart, and the increase in dosage be as follows : 0·015, 0·025, 0·035, 0·06, 0·08, 0·1 c.c. After 0·5 c.c. has been given, the dose should be given only once a week ; 0·1 c.c. is rarely exceeded. At the least sign of reaction the dose must be withheld until all reaction has disappeared, and it must not be increased until the patient fails to react to the dose which has once caused a reaction.

B.E. (human) and P.B.E. (bovine) are total cellular products, and consist of an emulsion in 50 per cent. glycerine of the pulverized bodies of the bacilli, without

prior removal of toxins soluble in distilled water. They contain 5 milligrammes of bacillary substance per c.c., and are probably the most active of all the preparations. The objection to them is the difficulty of absorption of the bodies of the bacilli, which may remain in the tissues, act as mechanical irritants, and cause a pseudo-abscess containing sterile sero-purulent fluid. The initial dose is 0·0005 c.c., and is repeated every second or third day, each time increasing the amount given by two or three times the amount previously given. When a reaction is produced, the injections are given at longer intervals—say every six to eight days. They are increased till 1 to 2 c.c. of the original is reached, but these large doses are given only every two to three weeks, on account of the slowness of absorption. In pyrexial cases the initial dose is not more than one-tenth of that indicated above. These preparations are the ones most favoured by Spengler, and are employed by him as follows :

Having determined the variety of organism at work, he gives an injection of a diagnostic dose of bacillary emulsion from the other variety of the bacillus. If the diagnosis is correct, the temperature, if elevated, usually falls, and the patient will declare he feels better ; if, on the other hand, the diagnosis is wrong, the remedy acts as a toxin, and the patient experiences an increase in the symptoms. When the patient feels better after the injection, the particular preparation is considered as the vaccine suitable to the case, and is used therapeutically. After one preparation has been used for some time, the other is employed till the patient is immunized to both strains. In proof of the antagonistic working of the two varieties of the tuberculin, Pottenger adduces the fact that if one preparation is injected, and proves to be the

toxin instead of the vaccine for the patient, a dose of the other may be injected at once, and will serve to counteract the toxic action of the first preparation (see Pottenger's 'Pulmonary Tuberculosis,' figs. 28*a* and 28*b*). The claim is made that this therapy is particularly valuable in febrile conditions and in tuberculosis of the larynx, kidney, or intestines.

*B. Immunization under the Guidance of the Opsonic Index.*

When the opsonic index is being employed as a guide to therapeutical inoculations, the assumption is made that the index has not altered materially between the time at which the blood-sample was taken and the presentation of the patient for inoculation after the determination of the index ; or if the procedure be to inoculate and take the blood-sample at the same time, the index is more or less guessed at from a study of the curve already secured. In many cases of pulmonary phthisis, and, indeed, in most where pyrexia is marked, the fluctuations are so marked, and the alterations in index so rapid, that in the interval between taking the blood-specimen and completing the index determination the blood-condition may have completely altered ; or, alternatively, it is quite impossible to calculate from a study of the chart what the index is likely to be at the moment of inoculation.

In such a case, to profess that treatment was being conducted under the guidance of the opsonic index would be a mere travesty of the truth. Tuberculin treatment must then be deferred until rest, physical and mental, partial or absolute, diet, and therapeutic measures, among which may be mentioned full doses of calcium salts, have caused the index to assume a more or less constant level,

or to move in a regulated manner. And here let me mention that, unless the index be determined to both human and bovine strains, as in Charts XVI., XVII., XVIII., the work is incompletely done, and a momentous change, such as is shown in Chart XVIII., may well be missed.

When the index estimated morning and evening for several consecutive days has remained more or less steady, a minimum dose of the selected tuberculin is given. For this purpose 0.00001 c.c. of either T.R. or B.E. of the human or bovine variety will suffice. The effect of this upon the index is then observed daily, or, still better, each morning and evening, in order to determine the length of the negative phase, if any occur, and to estimate the maximum response in index and the interval occupied in reaching the crest of the positive phase. A rise of 0.2 in the index attained in five or six days may be esteemed quite satisfactory, and be held to indicate the repetition of the dose in eight or ten days. Should no such rise occur, either the dose is too small or the variety of tuberculin inappropriate. In either case it is well to repeat the same dose, as sometimes this is followed by a much more marked response, as if the first dose had merely sensitized the organism to the tuberculin. The procedure indicated above is continued until such time as it is clear that the index moves in a regular manner, when estimations are performed at longer intervals. The object held in view is to cause a steady index at as high a level as possible. The dose of tuberculin is, however, only increased when the preceding dose fails any longer to bring about a satisfactory immunizing response. The increase in dosage is usually by 0.00001 c.c., and the maximum dose attained after six to twelve months' treatment may be taken to be 0.0001 to 0.0002 c.c.

Reliable and adequate statistics of the results achieved by this method of treatment are, unfortunately, lacking.

The difficulty of obtaining a steady index in pyrexial cases prior to beginning the vaccine treatment is great, the labour of estimating the indices, and the consequent expense to the patient, very considerable. Its scope in this condition is therefore limited. Apyrexial cases much more readily offer opportunity for treatment, but even in these it is no easy matter to secure the prior steady index.

Although it is a matter of doubt as to whether the opsonic index does really measure anything of value in immunity against the tubercle bacillus, in the treatment of a phthisical patient to whom expense is no object the value of the opsonic index to correlate with the temperature chart, physical signs, and symptoms, may be unhesitatingly admitted.

*C. Immunization conducted under the Guidance of Clinical Signs and Symptoms, and of the Lessons learnt from Opsonic Methods.*

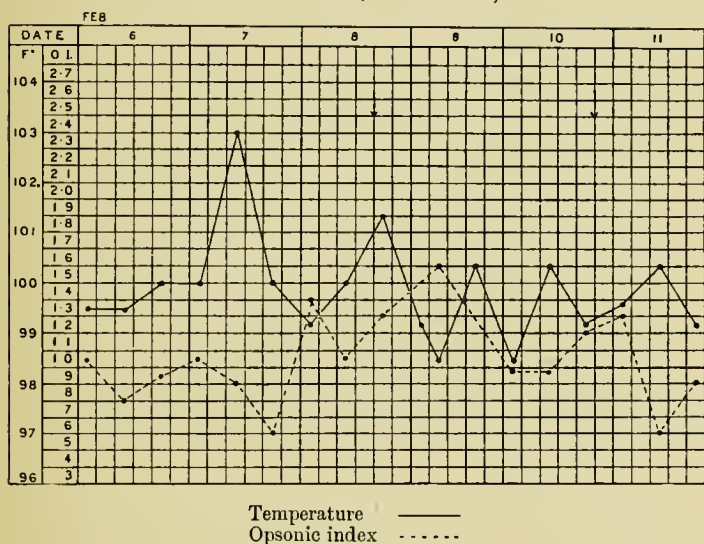
According to this method, while the assistance to be derived from determinations of the tuberculo-opsonic index is not necessarily altogether dispensed with, the main reliance in controlling the intervals and magnitude of the inoculations is placed upon observations of temperature, pulse, and clinical signs and symptoms generally.

The statement used to be made that temperature and pulse bore no relation soever to opsonic index. This has now been proved to be incorrect. Temperature and opsonic index vary more or less together. When the index is rising, the temperature is falling, and *vice versa*,



as is well shown in Chart XXI. Which of the two, temperature or index, affords the readier response to changes in immunity we know not as yet, but it is probable that alterations of temperature may be detected before any marked change has occurred in the index. The pulse-rate varies more or less closely with the temperature, and in the same direction, but is affected more readily by extraneous circumstances.

CHART XXI. (A. C. INMAN).



This chart shows how temperature and opsonic index were correlated in a case of pulmonary phthisis. The two will be seen to have moved together, but in reverse directions, a more or less constant phenomena.

Latham, Spitta, and Inman,<sup>1</sup> state that (1) when the temperature has been fluctuating from 97° F. to 98·4° F., and then remains level at 98° F., the opsonic index rises ; (2) when the temperature, previously level and normal or subnormal, rises to 99° F., the index falls ; (3) when the

<sup>1</sup> Proceedings of the Royal Society of Medicine, April, 1908.



temperature is persistently high, the index is persistently low ; (4) when the temperature, although remaining at about the same high level, fluctuates to a less degree, there is an improvement in the opsonic index.

The alterations in temperature, pulse, and opsonic index, are held to be due to the same cause—*i.e.*, auto-inoculations from the infected foci. These may be assumed to be more or less constantly going on when the disease is active ; but should they be too frequently repeated, or assume a magnitude greater than the ordinary, then the oscillations become the more marked. It thus follows that in apyrexial phthisis the auto-inoculations occur more or less regularly both in time and magnitude, while in pyrexial phthisis they occur irregularly both as regards intervals and size. In this latter case, if artificial inoculations are to be carried out, the necessity for restraining the irregular auto-inoculations by rest in bed, absolute if necessary, freedom from worry or excitement, and the various hygienic and therapeutic measures which clinical experience indicates, is sufficiently obvious. The attempt may be made to increase the coagulability of the blood by the administration daily of calcium lactate in sixty-grain doses.

Although the above is the view generally held as to the causation of pyrexia, especially if this be irregular in nature, I am convinced that it is only part of the truth. Tuberculosis usually causes little pyrexia unless it affect the meninges or be miliary in type. The admission of secondary infection to the tuberculous focus is almost certain to result in the onset of pyrexia, and what holds for tuberculosis elsewhere very probably holds for tuberculosis of the lungs ; and I cannot help feeling that it is to the action of the toxins of secondary organisms upon

nerve cells already sensitized by tuberculo-toxin that the production of pyrexia is largely due. This question of mixed infection will be more fully dealt with later.

If the guidance of the temperature chart is to be followed in the conduct of immunization, it is hardly necessary to point out that temperatures must be accurately taken. This is best done *per rectum*, in which a standard half-minute clinical thermometer should be placed for a full three minutes. Six-hourly temperatures should be taken—at all events, at the beginning of treatment—as an index as to what is really occurring, and as a guide for the various other therapeutic measures. I am, however, inclined to agree with those who hold that for immunizing purposes a chart should be prepared upon which the temperature is recorded only once a day. That taken at 9 p.m.—*i.e.*, one hour at least after going back to bed—has been recommended as the most useful.

Assuming that for a case of apyrexial phthisis the suitable exercise and mode of living has been determined, that in a case of pyrexial phthisis the temperature has been regulated by rest in bed, etc., and in each the variety of the bacillus discovered, it now remains to decide which tuberculin is to be utilized.

1. The theory of Spengler and Pottenger may be adopted, and their procedure followed for finding out which is the vaccine tuberculin, which the toxic one. The former of these will then be employed.

2. The ordinary method of using the human T.R. or B.E. for all cases may be followed.

3. The bovine T.R. or B.E. may be employed, as advised by Raw.

4. The mixed human and bovine T.R.'s or B.E.'s may be used, as recommended by the author two years ago.

This preparation certainly causes less rise of temperature than any other, and suits all cases : for if the infection be due to one type only, the variety of tuberculin which does not correspond to it may be assumed to be excreted, and can do no harm ; whilst if the infection be due to both types, then both varieties of tuberculin are requisite to meet the case.

5. The autogenous vaccine is to be prepared. Mean-time the variety of tuberculin corresponding to the infected type is employed, a polyvalent mixture being compounded of T.R.'s or B.E.'s from different sources. This would appear to be the most logical procedure.

A minimal dose of tuberculin—say 0·00001 c.c. for apyrexial cases, 0·000005 c.c. for pyrexial ones—is then given, the patient kept in bed for at least twenty-four hours, and the effect upon the temperature observed. If this be not affected, the same dose should be repeated in about seven days ; for it must not be forgotten that a second dose may produce a marked change, as if the cells had been sensitized by the first. A rise of 1° F., followed by a speedy fall to a lower level than previously, is to be accounted a satisfactory response, as it is also if the temperature fall without any precedent rise. A sudden elevation of a couple of degrees or a steady rise for two or three days is the signal for a fresh inoculation, the dosage being increased only when that being used fails to produce any effect upon the temperature. The increase will thus be very gradual, and at the end of nine to twelve months a dose of 0·0001 c.c. to 0·0002 c.c. only may have been attained. In apyrexial cases, when the temperature remains steadily near the normal, intervals of about ten days will probably be found satisfactory.

To formulate any definite scheme which will suit all cases is quite impossible, and the above is but a mere outline for procedure. Modifications will certainly be needed to suit the individual patient. Close clinical observation and common-sense will indicate these.

#### IMMUNIZATION BY MEANS OF CONTROLLED AUTO-INOCULATION.

Guided by the deduction that the tuberculin best suited for any case is that elaborated by the patient himself, it occurred to certain observers to turn to good use the ill-arranged auto-inoculations which we have seen occur in many cases of pulmonary tuberculosis. The patient is put to bed, and absolute rest enjoined, if necessary, until the temperature has been reduced to normal, or been steadied at a level slightly above it. A rise of temperature to 99° F. plus headache as a result of work is regarded as indicating a condition of hyper-inoculation calling for absolute rest. By means of forced respiration through a bottle of water or a Kühn's aspiration mask, the patient is obliged to do work and auto-inoculate himself. Patterson<sup>1</sup> secures the same result by means of exercise, and later by manual labour, under the guidance of temperature and clinical signs carefully graduated.

It is not, however, possible to exercise complete control over the dosage in all cases. Some, moreover, fail to auto-inoculate themselves from the beginning, while, if improvement occur, it is obvious that after a certain phase has been reached inoculation by gradually diminishing doses alone can be induced. It would also appear that a lasting and efficient immunizing effect is by no means

<sup>1</sup> *Lancet*, January 25, 1908, p. 216.

always obtained, and the aid of tuberculin from without must be sought. For full particulars of this method, the reader should consult the papers by Patterson and Hort.

### RESULTS OF VACCINE THERAPY IN PULMONARY TUBERCULOSIS.

#### *A. By Methods controlled by Clinical Symptoms.*

Pottenger,<sup>1</sup> after pointing out that culture products are to be used in *tuberculosis*, and that they are not represented as having any influence over dead tissue, or as being able to regenerate cells that have been destroyed, and therefore that their proper sphere is in incipient cases before mixed infection or breaking down with absorption occurs, gives the following statistics of the comparative results obtained in first-stage tuberculosis by sanatorium methods pure and simple, and by sanatorium treatment supplemented by tuberculin therapy according to the methods outlined on p. 211 :

Four observers treated 611 first-stage cases with all the advantages of sanatorium treatment, and apparently cured 391, or 64 per cent.

Ten observers supplemented sanatorium treatment with tuberculin therapy in 589 similar cases, of which 496, or 84·2 per cent., were apparently cured.

He considers that these are quite sufficient cases upon which to base an opinion, and considers that culture products certainly stand the test and accomplish that for which they are recommended—namely, the cure of pure tuberculosis.

<sup>1</sup> 'Pulmonary Tuberculosis,' p. 343.

## TRUDEAU'S RESULTS.

From 1890 to 1901 Trudeau in America employed inoculation treatment in cases of pulmonary phthisis. The adjoined table is a comparison of the results obtained by pure sanatorial measures with those supplemented by inoculations of tuberculin.

TABLE XVII.

(1) Cases treated (Non-Tuberculin).	(2) Cases treated with Tuberculin.	Advantage to (2) over (1).
1,367	143	
Alive, 38·0 per cent.	Alive, 58·0 per cent.	20·0 per cent.
Dead, 36·6       ,,	Dead, 33·0       ,,	3·6       ,,
Balance untraced		

## INCIPIENT CASES ONLY.

(1) Cases treated (Non-Tuberculin).	(2) Cases treated with Tuberculin.	Advantage to (2) over (1).
Alive, 61·0 per cent.	Alive, 76·7 per cent.	15·7 per cent.

Turban's results at Davos Platz<sup>1</sup> have been as follows :

In the first stage his results were such as led him to say : ' Now, if we compare the results in early cases in which tubercle bacilli were found in the sputum, the result is substantially in favour of tuberculin treatment.

' Of cases in the second stage 48 were treated with tuberculin ; 9 died within two years of treatment ; 3 died within three years of treatment and more ; 16 were alive six years after treatment ; 5 more alive five years after treatment ; 3 more alive four years after treatment. In all, 36 were alive. Thus, of 48 cases 36 were alive and

<sup>1</sup> *Weicker. Beit. z. Frage der Volksheilstaetten*, p. 22.

12 dead. Of 152 cases treated in the ordinary way, 45 were dead and 107 alive ; but the figures show greatly in favour of tuberculin treatment, because of the 107 alive a great number (49) were under treatment more than one or two years.

‘ Of cases in Stage III., 21 cases were treated with tuberculin. In 8 there was tuberculous laryngitis ; 9 cases survived five years ; 3 more survived four years. Only 5 out of 21—or 25 per cent.—died within two years ; 3 were quite well six years after. In all 5 were well.

‘ Of cases treated in other ways, 61 out of 84 died, and of these, 41—or nearly 50 per cent.—died within two years. Compare this with 25 per cent. under tuberculin treatment.’

Thus, even in the second and third stages of the disease, which do not strictly fall within the zone of tuberculin treatment, a great advantage is seen to exist on the side of vaccine therapy.

*B. Results achieved under the Control of the Opsonic Index, or according to the Methods which Experience in Opsonic Work has indicated.*

The opsonic method has been upon trial for far too short a time to enable anything like reliable statistics to be collected. Nothing more than the impressions gained as to its value by different observers can be adduced in its favour. The most complete series of cases so far published are those of Turton,<sup>1</sup> who describes the results obtained in 26 cases of tuberculosis of lungs and pleura, in 19 of which control by the index was employed.

<sup>1</sup> ‘ International Clinics ’ (eighteenth series), vol. ii., p. 23.



In 6 early cases without great lung destruction or severe constitutional disturbance the disease was apparently arrested, there being no abnormal physical signs or symptoms for six months. Their gain in weight respectively was 19, 13,  $17\frac{1}{2}$ , 3,  $12\frac{1}{2}$ , 14 pounds.

In 6 cases, of which 2 were far advanced, with great loss of weight and constitutional disturbance, great improvement occurred. Symptoms, however, were still present, or six months had not elapsed since apparent cure. Their gain in weight respectively was  $35\frac{1}{2}$ , 13, 17, 7, 23, and 38 pounds.

In 6 cases there was some improvement in general health, and the signs and symptoms were ameliorated, but the result was not entirely satisfactory. These were most severe cases of long standing, with considerable lung destruction or some complication, their gain in weight respectively being 3,  $3\frac{1}{2}$ , 0, 5, 8, 0 pounds.

In 3 cases there was little or no improvement, but they were very unfavourable cases, the home surroundings being very unsatisfactory, disease extensive, and general health rapidly failing.

Five cases died either during or after suspension of treatment, but were practically hopeless from the first, and were only injected as a last resort.

All these cases were treated out of hospital, in some cases with very unsatisfactory home surroundings; and almost all had to carry on their usual occupations. Besides the tuberculin, cod-liver oil and occasional tonics were the only remedies used.

His doses varied from 0·0001 to 0·001 c.c., chronic cases having the larger doses at the longer intervals—say 0·0005 c.c. every twelve to twenty days—while acute cases did better on a dose of 0·0002 c.c., repeated in from

seven to twelve days. It cannot be too strongly emphasized that, if an accurate estimate is to be formed of the true value in pulmonary tuberculosis of very small doses of tuberculin administered at long intervals, with or without the guidance of the opsonic index, observers must divide their cases up methodically into classes, as is done by Trudeau, Pottenger, Turban, and others, and the subsequent histories of the cases carefully watched for a term of years.

#### SECONDARY INFECTION IN PHTHISIS.

It is to the comparative inattention paid to the mixed infection in pulmonary tuberculosis that the poor results obtained by specific treatment in the more advanced stages is to be attributed. How great the natural powers of resistance to the tubercle bacillus are can be gauged from the high percentage of the human race who become infected in infancy and youth, and yet recover spontaneously. Tuberculin therapy cannot possibly influence mixed infection, which, as Pottenger points out, exists in cases displaying fever of even mild degree, and has doubtless an influence on the tuberculous process in many cases where no rise of temperature exists. Personally I should be inclined to go almost so far as to say, Exclude secondary infection, and every case of pulmonary tuberculosis would recover if placed under suitable conditions. A simple experiment on the lines of that mentioned on pp. 174 and 175 will show the importance of a mixed infection. Take a uniform emulsion of living human tubercle bacilli, and add equal volumes of emulsion—say 0.1 c.c. to 10 c.c. of glycerine broth in each of two culture-tubes, A and B. Prepare a broth

culture of *Staphylococcus albus* from a case of pulmonary phthisis, and after twenty-four hours' incubation sterilize at 60° C. for one hour. Now to tube A of tubercle in glycerine broth add 2 c.c. of this sterile staphylococcic broth, and place the two tubes, A and B, to incubate at 37° C. At the end of a week little visible growth will have occurred in tube B, whilst profuse growth may be seen in tube A. The staphylococcic broth has acted as a manure to the tubercle bacilli, and promoted their multiplication. It would thus appear probable that mixed infection has a twofold action. Not only does it lead to softening and disintegration of the lung tissue, but it may also increase the rapidity of growth of the tubercle bacilli.

It was realization of some such connection as this between the two that induced me to devote the past five years to a study of the catarrhal organisms of the respiratory tract. It was, however, only recently that I was able to demonstrate its truth *in vitro*. Not all the micro-organisms which occur as secondary infection perform this function towards the tubercle bacillus, and I am at present engaged in determining which do and which do not, and what precise part the latter play.

In addition to the catarrhal organisms, and excluding *Bacillus septus*, mentioned in Chapter X., the following are commonly met with in the lung : *Streptococcus longus*, *Staphylococcus albus* and *aureus*, and occasionally *Bacillus coli* and the *Micrococcus tetragenus*. The last of these has, however, I think, been confused with the *Micrococcus paratetragenus* more often than not. Until fuller investigation has been made, the order of importance of these secondary invaders may be tentatively arranged as follows: Staphylococcus, Pneumococcus, Streptococcus, *Bacillus coli*, *Micrococcus catarrhalis*, *Bacillus influenzae*, *Micrococcus*

*tetragenus* and *paratetragenus*, *Bacillus* of Friedländer. Usually two at least of these are to be found acting in conjunction.

Special precautions are necessary to facilitate the determination, in a specimen of tuberculous sputum, of the organisms which come from the lung proper, as distinguished from contaminations picked up during the expulsion of the sputum. The following directions will help: Immediately on waking in the morning, the patient should brush the teeth thoroughly with water that has been well boiled overnight, and set aside in a covered vessel. The mouth is to be well rinsed out, the throat gargled, and a couple of mouthfuls of the sterilized water swallowed. One cough should be given, and the specimen of sputum be expelled directly into a sterile wide-necked bottle. If a nummular piece of sputum has been secured, it should be washed in sterile water, placed in a platinum or aluminium spoon, quickly heated for a few seconds over the Bunsen flame, then turned over and the heating repeated. It is then cut open with a sterile knife, and specimens taken from the centre to plant upon blood-agar plates, and for direct examination after staining by Gram's method and counterstaining for forty-five seconds with an aqueous 1 per cent. solution of neutral red. Such bacteria as are to be seen inside bronchial or polymorphonuclear cells can safely be assumed to have had their habitat within the lung, whilst the error introduced by assuming that this holds for all the varieties present in the specimens prepared in this manner is not considerable. After twenty-four hours' incubation the blood-agar plates are examined, and the various bacteria subcultured. If thought advisable, opsonic index determinations may be made to ascertain against which of these the body is failing to immunize

itself. Personally I do not often do this, but look upon all the bacteria present as invaders, against the attacks of whom it is well to assist the body.

If the sputum be not nummular, extra care must be taken in rendering the mouth and throat as sterile as possible.

From each of the bacteria isolated a vaccine is prepared in the usual way. These may all be mixed together, the strength of each being adjusted as thought fit, or be kept separate.

Immunization against the secondary infection may be conducted at the same time as tuberculin treatment ; but if it be, it is very difficult to determine how much of any temperature reaction is due to the tuberculin, how much to the vaccine. It is better procedure, perhaps, on this account to administer each separately on alternate weeks. Personally I prefer to begin treatment with the vaccine while the patient is undergoing preliminary rest in bed, and find that the reduction of pyrexia is thereby facilitated in many cases.

Of the ultimate results achieved by this therapy it is, perhaps, too soon to speak ; but in no uncomplicated case which has passed through my hands during the past four years has a course of six months' treatment failed to produce marked improvement, and of my cases nearly half were old-standing cases which had tried sanatorium treatment, and every accessory within their reach, both in England and abroad, and had failed to improve beyond a certain point. Encouraging though this be, I feel it unwise to be too optimistic.

Pottenger, after treating a number of cases upon these lines, came to the following conclusion :<sup>1</sup> ' I do not doubt

<sup>1</sup> ' Pulmonary Tuberculosis,' p. 261.

but that the true remedy will be obtained in a vaccine made from the cultures taken from the strain of the micro-organism found in each individual patient. The results which we have had so far in our endeavours to treat in this manner are very encouraging.'

Bonney<sup>1</sup> gives a detailed account of his results in fifteen cases, which are especially interesting in as much as 'no patient has been included in this group concerning whom there could have been entertained any rational hope of improvement other than upon the basis of an early control of the septicæmia.' 'The predominant mixed infection was in five instances *Staphylococcus aureus*, in two cases *Staphylococcus aureus* and *albus*, in one case *Staphylococcus albus*, in three cases *Streptococcus*, in two cases *Pneumococcus*, in one case *Colon Bacillus*, and in one *Micrococcus catarrhalis*.' The endeavour was made to control inoculations by opsonic index determinations, but this he found unsatisfactory, the tuberculo-opsonic index being higher and more constant than had been expected, in many cases remaining strangely in the vicinity of normal; whilst the indices to the secondary infection did not exhibit the violent fluctuations one might have anticipated from so severe a systemic invasion. This has been the result of my own observations also. Although Bonney confesses his inexperience in work of this nature, and his consequent employment of injudicious dosages, he says: 'From a clinical aspect, the evidence was conclusive that in some instances much good resulted from the employment of bacterial vaccines.' Again: 'The results in some cases have been uniquely satisfactory, while in others the improvement has been slow and somewhat disappointing.' Again, of thirteen of the cases

<sup>1</sup> 'Pulmonary Tuberculosis,' p. 749.



reported in detail, he says : 'Ten exhibited very substantial improvement. The condition was so desperate in each instance, and the improvement so conspicuous, as to justify an assumption concerning the efficacy of the specific treatment.' As initial doses, he found it best to use 5,000,000 streptococci, 10,000,000 staphylococci, 5,000,000 pneumococci, 50,000,000 *Micrococcus catarrhalis*. His final conclusion<sup>1</sup> is that bacterial vaccines present possibilities of benefit far beyond the limits of former therapeutic efforts, and that discriminating clinical study is essential in the determination of the size and frequency of dose.

#### VACCINE THERAPY IN OTHER FORMS OF TUBERCULOSIS.

1. *Tuberculous Adenitis*.—Of the frequency of tuberculous adenitis, often unrecognized in children, there is little need to dilate. Thus, MacConkey and MacFadyen found virulent tubercle bacilli present, usually in the mesenteric glands, of about 25 per cent. of children who died from non-tuberculous causes. In infants dying of tuberculosis, Steiner and Newieter found the lymph glands affected 299 times in 302 post-mortems, the bronchial glands being involved 286 times. Rilliet and Barthez found lymphatic glands involved 248 times in 312 cases ; and Northrup every time in 125 cases. Not only are the glands involved, but they are the first to show the disease in a large majority of cases, if this can be inferred from the fact that the glands show the most advanced processes. All cases of tuberculosis of the glands, however, do not show tuberculosis elsewhere, nor are all enlarged lymphatic glands tuberculous (about 60

<sup>1</sup> *Loc. cit.*, p. 762.



per cent. are). Steffen, however, says : ' Healthy lymph glands are not attacked by tuberculosis. They are predisposed thereto when they are swollen, succulent, and infiltrated, and in a condition of hyperplasia.' While Osler says : ' A special predisposing factor in lymphatic tuberculosis is a catarrhal inflammation of the mucous membranes, which in itself excites a slight adenitis.'

The extreme importance of putting all children, and especially infants, under the best hygienic conditions is thus obvious, while the advisability of applying the tuberculin test, best by means of Pirquet's cutaneous reaction, in all cases where glands are chronically enlarged, the nutritional condition bad, and the child unduly pale or peevish, deserves earnest consideration, in view of the fact that a large percentage of those who have enlarged glands during childhood develop tuberculosis in later life.

*Variety of the Bacillus Responsible.*—This has been determined in fifty-eight cases, with the following result : The bovine variety was found in thirty-three cases, the human in twenty-four, and both in one. In so far as one can deduce from so few cases, it thus appears that the bovine is rather the more frequently found variety. It is obvious that if either a T.R. of human origin or that of bovine origin be employed invariably, it will, strictly, be the most appropriate tuberculin in only about 50 per cent. of the cases. It therefore becomes necessary either to determine which is the right tuberculin, according to one or other of the methods previously outlined, or to adopt the suggestion of the author, and always employ a mixture of the two T.R.'s in equal proportions ; this latter is the less scientific, but is perhaps the more easily workable scheme.

Once the diagnosis has been made that enlarged glands

are tuberculous, it becomes incumbent not only to place the child under the best possible hygienic conditions, but also to submit it to a course of tuberculin treatment, in view of the satisfactory results thereby obtained, and so prevent subsequent caseation and sinus formation, and perhaps pulmonary or some other serious form of tuberculosis.

When caseation is present, prolonged treatment may be necessary, and perhaps surgical interference. If liquefaction be a feature, an extensive operation may be sometimes obviated by the preliminary raising of the index to 1 or over, and the passage of a tenotome into the caseous mass and the expression of the contents.

Sinuses may be aided by the additional treatment of a vaccine of the secondarily infecting organisms, scraping, and perhaps the application of hypertonic solutions of common salt and citric acid, to assist transudation of lymph rich in protective substances.

Wright, White,<sup>1</sup> Western,<sup>2</sup> Rivière,<sup>3</sup> Loveday and Ramsbottom,<sup>4</sup> and many others, have published satisfactory results, despite the sole employment of T.R. of human origin, which, as said before, cannot have been always appropriate. Carmault Jones<sup>5</sup> analyzes all the cases treated at St. Mary's Hospital with human tuberculin during the preceding five years. The cases as a rule were bad ones, and, being in out-patients, the treatment could not be carried out under ideal conditions. It would appear that, in any eight cases treated by inoculation, five are to be expected to make marked improvement, two or three of these being

<sup>1</sup> *Journal of Medical Science*, Dublin, September 2, 1907, p. 161.

<sup>2</sup> *Lancet*, November 23, 1907, p. 1449.

<sup>3</sup> *British Medical Journal*, October 26, 1907, p. 1131.

<sup>4</sup> *Medical Chronicle*, June, 1908, p. 145.

<sup>5</sup> *British Medical Journal*, August 28, 1909, p. 531.

cured ; two will improve slightly, and one or two will fail. The best results are to be anticipated in young children under ten, and in young adults from fifteen to twenty-five years old, after which they become gradually less favourable. The period of puberty, between ten and twenty-five, yields the worst results. Table XVIII. expresses the results in percentages :

TABLE XVIII. (CARMAULT JONES).

Result.	Years of Life.					
	1st Five.	2nd Five.	3rd Five.	4th Five.	5th Five.	6th Five.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Good .. ..	75	50	50	84	70	43
Moderate ..	25	44	20	0	7	43
Bad .. ..	None	6	30	16	23	14

2. *Tuberculosis of Bones and Joints.*—Far too few observations as to the variety of bacillus at work have as yet been made to enable any definite conclusion to be drawn, but it may be noted that in the instance of all ten cases cited on p. 185 the human variety was found.

For a case of early joint disease, splints, rest, Bier's congestion, and tuberculin, will probably suffice to effect cure. Should the disease be advanced, and the surgeon decide upon scraping or excision, a preliminary raising of the resistance by means of tuberculin will minimize the risk of dissemination, and a continuation of such treatment after the operation will expedite the cure. It may, however, be noted that cases of this kind, so advanced that even amputation was advocated, have cleared up in such a marvellous manner under tuberculin and the usual therapeutic measures that no case need be considered hopeless until such measures have received trial (see Chart XXII. for instance of such a case).

Secondary infections will, of course, require their appropriate vaccines.

Western (*loc. cit.*) gives his results in twenty-six cases as follows :

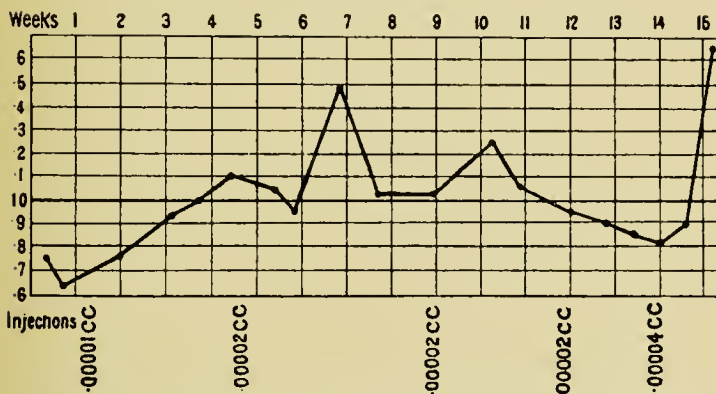
Fourteen cases were cured.

Five cases showed marked improvement, and were still under treatment.

Two cases showed slight improvement.

CHART XXII. (DR. J. W. E.).

SEVERE TUBERCULOUS SYNOVITIS OF KNEE. FIRST FOUR MONTHS OF TREATMENT.



Fluid from joint when inoculated into a guinea-pig produced typical generalized tuberculosis in eight weeks. Complete cure in six months, with perfect mobility of joint.

Five cases showed no improvement, but two of these were senile cases in patients over sixty.

In young subjects and early cases cure and good movement may be expected ; but where there is much destruction of tissue and sinuses are present, progress is slow and movement may be considerably limited.

Raw<sup>1</sup> records his experience in twenty-seven cases,

<sup>1</sup> *Lancet*, February 15, 1908, p. 480.

mostly of a chronic or subacute variety : ‘The cases where the best results were obtained were those in which there was some suppuration or sinus leading directly down to tuberculous disease. In cases of pulpy disease of the joints there was, in many instances, marked diminution in the size of the joints, with absence of inflammation and more movement.’

Turton treated five cases, one being of the elbow, the other four spinal. The former was completely cured. Of the spinal cases one had had three operations, and there were sinuses in the back, loin, and iliac region. Despite secondary infection with *Staphylococcus albus*, the patient did extremely well, gained 2 stone in weight, and has been at work for over a year. In the second, an early case, the disease was apparently arrested. The third was an old case, and was only under treatment a short time, when improved general health resulted. The last case was hopeless from the first, and died of lardaceous disease.

Painter<sup>1</sup> reported on eleven cases as follows : He excludes two cases as being unfair. The other nine were all advanced cases, usually with mixed infections. Two cases were extraordinarily improved, one considerably so ; four not at all. He considers the method worthy of a longer trial, and believes that with a more judicious selection of cases better results would be forthcoming.

In my own experience these cases do extremely well, especially if treatment be simultaneously directed against any secondary infection ; the cases which do not do well are those in which other parts are also affected, where there is marked wasting or signs of lardaceous disease, or where secondary infections cannot be controlled owing

<sup>1</sup> *Boston Medical and Surgical Journal*, October 31, 1907, p. 621.

to neglect on the part of the patient. Chart XXIII. exhibits the fluctuations in opsonic index in a case of caries of the superior maxillary bone undergoing tuberculin treatment with indical control of the injections.

A summary by Maynard Smith of the results obtained at St. Mary's Hospital during the past few years is to be found in the *Lancet* of August 14, 1909, p. 470. They will be seen to have been highly satisfactory. This, I cannot help feeling, is largely due to the fact that a tuberculin of human origin would appear to be the appropriate one.

#### LUPUS AND OTHER TUBERCULIDES OF THE SKIN.

In no other branch of tuberculin therapy have such absolutely discordant results been obtained. Upon the one hand, we have the following opinion of Reyn and Petersen :<sup>1</sup> ' The results of the sole treatment with T.R. have been very bad. We could not in any of the cases see any improvement at all following treatment according to Wright's method. In the most fortunate cases the affection remained stationary ; in most of the cases it became worse. We wish especially to call attention to the fact that in two out of the three early cases the disease spread remarkably quickly during the T.R. treatment. We therefore feel bound to dissuade practitioners from employing this method as a sole treatment for lupus vulgaris, both in old cases and in fresh apparently benign cases.'

Upon the other hand, Raw :<sup>2</sup> ' Twenty-four cases of lupus have been treated, and it is here that the very best results of tuberculin are obtained. All stages of the disease seemed to respond rapidly to injections, and in

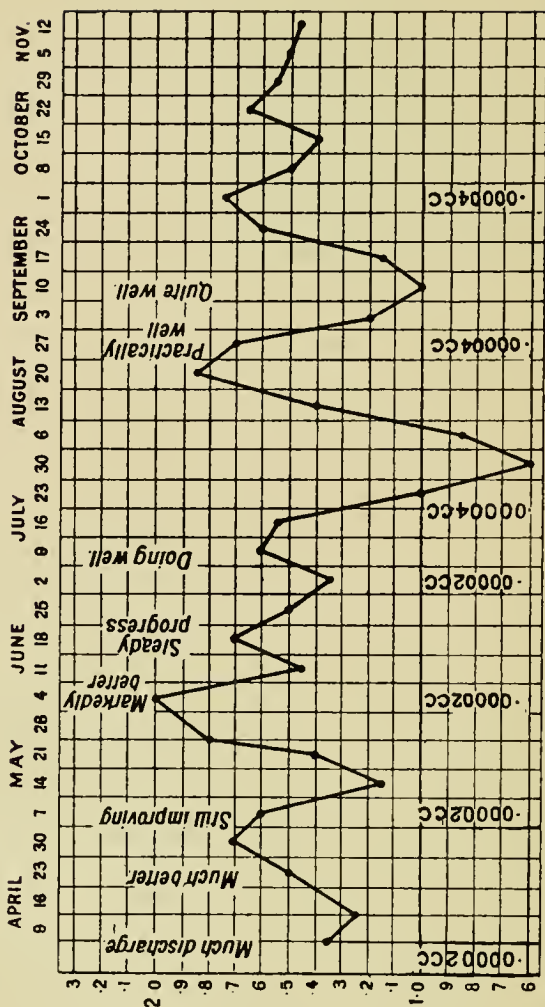
<sup>1</sup> *Lancet*, April 4, 1908, p. 1004.

<sup>2</sup> *Ibid.*, p. 481.



## CHART XXIII.

CASE OF CARIES OF SUPERIOR MAXILLARY BONE. PURE TUBERCULAR INFECTION.



It will be noticed that the injections of 0.0004 c.c. on July 23 and August 27 produced very great fluctuations in the index, indicating that these doses were unnecessarily large. The patient, however, continued to do extremely well, and on September 10 no sign of the infection remained beyond a slight scar. The considerable reaction of index to injection, however, indicated that in all probability tubercle bacilli were still present. Injections of reduced doses were therefore continued until the index remained practically steady.



all the cases in which I have used tuberculin without any other treatment, such as scraping or medication, the ulcerated surface has healed with a firm cicatrix, and in only two instances so far has the disease recurred.'

Between these two diametrically opposed opinions are to be set the experience of Wright and of Bulloch and Western.

Wright finds that in those varieties of lupus where the skin is dry and scaly—so-called 'lupus psoriasis'—tuberculin is of little avail; while in suppurating lupus, where mixed infection by the *Staphylococcus albus* is present, good results can only be achieved by a simultaneous attack upon the secondary infection.

Carmault Jones (*loc. cit.*), analyzing the 23 cases treated at St. Mary's by inoculation during the past five years, gives the following results: Cured, 3; much better, 8; better, 9; unchanged, 1; unknown, 2. Relapse occurred five times. The average duration of treatment in successful cases was two years. The initial dosage is about 0.00002 c.c. T.R. or B.E., and a maximal dose of 0.0001 or 0.0002 c.c. may be necessary.

Bulloch and Western also find that the ulcerative type does much the best with tuberculin, especially if combined with fomentations. Treatment may have to be very prolonged, and is best continued long after disease has apparently disappeared, as fall of index seems to predispose to a renewed attack.

Whitfield's results have been disappointing.

The proper rôle for tuberculin in lupus would appear to be as an adjuvant to actino-therapy and surgical measures.

Western (*loc. cit.*) records the successful treatment of two cases of erythema induratum, one presenting ulceration, the other not.

Whitfield<sup>1</sup> finds that tuberculous ulcers have done well, even though in some cases the surroundings have been as adverse as possible. Of two cases of Bazin's disease, one apparently completely recovered; the other, after improving enormously at first, relapsed, and seemed to derive little benefit from further treatment.

#### TUBERCULOSIS OF THE GENITO-URINARY SYSTEM.

Pardoe<sup>2</sup> lays stress upon the frequency and non-recognition of this form of tuberculosis, and especially of that of the bladder. So disappointing have been the results of operative treatment and of all kinds of bladder washes and instillations that he declared that he himself had never met with a case even of apparent cure of vesical tuberculosis by such means. Tuberculin treatment has here met with success that can only be called brilliant. Pardoe himself treated twenty-one cases with tuberculin, many of these before opsonic work was known. Despite this fact, and the certainty, as he himself admits, of having at times given much too large doses and at improper intervals, he obtained the following results :

TABLE XIX.

	Per Cent.		Per Cent.
Cured 5 cases out of 21 ..	24	No improvement in 6 cases out of 21 .. ..	28
Greatly improved 4 cases out of 21 .. ..	19	Death in 6 cases out of 21	28
	—		—
	43		56

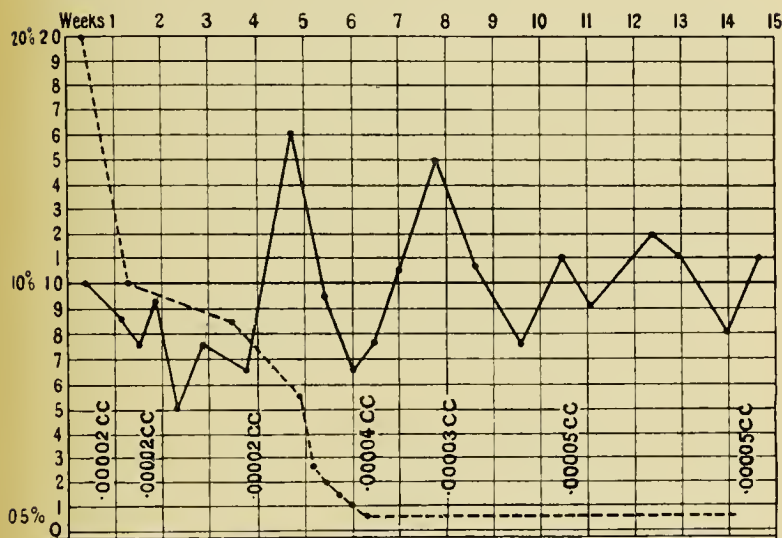
He, however, considers that tuberculin should never be given in genito-urinary cases if the orifices of both ureters are infected.

<sup>1</sup> *Practitioner*, May, 1908, p. 697.

<sup>2</sup> *Lancet*, December 16, 1905, p. 1766.

Other observers whose work has been guided by opsonic determinations do not agree with this, and have obtained even more encouraging results (Chart No. XXIII., a case under Dr. Eyre, affords an especially good example of this). Thus, Western (*loc. cit.*) says that where

CHART XXIV. (J. W. E.).



Dotted line = percentage of pus in urine.  
Continuous line = tuberculo-opsonic index.

This chart exhibits the first four months' result of treatment in a very bad case of renal and vesical tuberculosis. The cystoscope showed advanced disease of the right kidney, slightly earlier in the left kidney, and advanced in the bladder. The patient improved greatly under treatment, and was alive and in fair health two years from the commencement of treatment.

adequate treatment has been carried out good results have followed. In one case with slight ulceration of the trigone, pain and frequency of micturition, with blood and bacilli in the urine, and a hard, nodular prostate, all urinary symptoms disappeared in six months, though the prostatic condition remained.

Turton (*loc. cit.*) treated 4 cases of tuberculous kidney; 1 died, the other 3 were greatly improved, and, in Turton's opinion, derived very great benefit from the injections, especially as in 2 of these all the resources of surgery had been exhausted.

Western (*loc. cit.*) records 15 cases of tuberculous epididymitis. Of these, 6 were cured in from three to nine months; 4 were greatly improved, the sinuses healed, and though the disease was still evident, the patients were able to go about; 4 had only been under treatment less than two months, but were doing well, and 1, which also suffered from diffuse pulmonary phthisis, alone derived no benefit. Western concludes that good results may be expected, even when the disease has broken down and sinuses are present.

For two cases of advanced genito-urinary tuberculosis apparently greatly benefited by tuberculin treatment, see Bonney (*loc. cit.*, p. 509).

The results obtained in thirty-four cases at St. Mary's Hospital may be thus tabulated :

TABLE XX.

Site of Infection.	Total Number.	Cured.	Much Better.	Better.	No Better, Worse, or Dead.
Testes ..	19	3	2	2	2
Kidney ..	8	2	2	3	1
Bladder ..	17	2	4	8	3

The initial dose is 0·00001 c.c., and doses larger than 0·00005 c.c. are rarely employed. When the disease is so advanced as to be beyond the efforts of the surgeon, tuberculin treatment *can be unhesitatingly recommended as holding out the only hope of amelioration and of prolonged life.*

The importance of paying due attention in these cases to secondary infections, especially by the *Bacillus coli communis*, can hardly be overestimated.

#### TUBERCULOSIS OF THE INTESTINES.

Very little use appears to have been made of tuberculin therapy in this condition. Turton (*loc. cit.*) records the following remarkable case: 'The abdomen was opened, and such extensive disease found of the cæcum and ileum that removal was impossible. Thirty-eight injections of T.R. and twenty-one of B.C.C. vaccine were given in sixteen months, with the result that the patient gained 16 pounds in weight, and the tumour, which was the size of a goose's egg, entirely disappeared. The patient has been back at work over a year, and has only very rare attacks of pain very mild in character.'

#### TUBERCULOUS PERITONITIS.

Here excellent results have been obtained, both with and without laparotomy. The best procedure appears to be preliminary raising of the index with T.R., laparotomy, and, after allowing the full effects of the auto-inoculation induced by the operation to pass off, resuming tuberculin treatment. Raw (*loc. cit.*) records his results in eight cases, four being of the dry plastic variety, four associated with fluid in the abdomen. All recovered, and were discharged well after twelve inoculations. He considers that tuberculin is an absolute specific in cases where the disease is confined to the abdomen. Wright<sup>1</sup> gave the following details of a most interesting case: Laparotomy had been performed two months previously, without any

<sup>1</sup> *Clinical Journal*, November 9, 1904.

resultant improvement. A temperature of 100° F. persisted, the wound discharged, and the condition became desperate. Under T.R. the temperature came down to normal in six weeks, and in three months the patient was discharged. In six months the gain in weight equalled 27 pounds, and six months still later the patient was seemingly perfectly well.

#### TUBERCULOUS MENINGITIS.

Even in this extremely grave form of tuberculosis successes have been reported. It is not to be supposed that there have not been failures, but, on the other hand, some successes also have not yet been published.

Buchanan showed before the Liverpool Medical Institute on December 5, 1907, a child who had suffered from tuberculous meningitis, and recovered after an illness of four weeks, with coma for eight days. The symptoms were classical, Calmette's test reacted positively, and the cerebro-spinal fluid contained excess of lymphocytes. An injection of 0.000025 c.c. T.R. was given; the child showed almost immediate improvement, and gradually regained consciousness. Three weeks later the dose was repeated. Recovery was uninterrupted, and the child was quite intelligent and able to run about.

Raw (*loc. cit.*) relates the following account of four cases, which exhibited all the classical symptoms: In two, tuberculin had no effect, and the children died; in the other two, all the symptoms disappeared after four injections, and the children made a rapid recovery.

#### OCULAR TUBERCULOSIS.

My experience in this form of tuberculosis, whether of the choroid, iris, or cornea, has been uniformly favour-

able during the past three years—*i.e.*, since the adoption of mixed human and bovine T.R. as the immunizing agent. Cases for which clinical experience would have indicated a course of six or nine months' treatment with human T.R. have recovered instead in four or six. Two cases—one of tuberculous choroid and cornea, the other of episcleritis, with tubercular glands—which were mentioned in the second edition as making exceptional progress, the improved general condition and increase in weight being also very marked, soon made a complete recovery.



## CHAPTER XII

### VACCINE THERAPY IN EYE DISEASES

OPHTHALMIC surgeons would probably be the first to admit that little further progress in ophthalmology is to be expected from surgery pure and simple. The prime essential is increase of knowledge in the pathology of such conditions as trachoma, Mooren's ulcer, spring catarrh, and sympathetic ophthalmia. Should a bacterial origin be established for these, treatment by vaccines should hold out considerable promise of success.

The scope for vaccine therapy in diseases of the eye is already great, and is steadily increasing. The first essential for its success is accuracy in the determination of the infecting organism or organisms. In cases of doubtful tuberculosis the opsonic index does not always help ; how variable it may be is shown in Table XXI.

The explanation of this is that the tendency in bacterial diseases of the eye is for the index to be raised. In tuberculosis, however, the ocular infection is often complicated by chronic infection elsewhere, either of the glands, lungs, or bones, which tends to the production of a lowered index. These two factors, working in opposite directions, may result in an index within the normal limits.

The discovery of Calmette's ophthalmo-reaction (p. 194) promised to be of great service. Thus, Brunetière<sup>1</sup> has recorded its value in discriminating between interstitial

keratitis due to syphilis and to the tubercle bacillus. Anbault and Lafon<sup>1</sup> obtained positive reactions in a case of solitary tubercle of the choroid, in two of phlyctenulæ, in episcleritis, in tubercular interstitial keratitis, and in optic neuritis with a choroidal nodule; also in four cases of healed phlyctenule. Stephenson<sup>2</sup> has employed it in over thirty cases, among which were six cases, in children, of relapsing ulceration of the cornea. A positive result

TABLE XXI.

Case.	Nature of Cases.	Index.
1	Interstitial keratitis .. .. .	2·2
2	Phlyctenules .. .. .	1·9
3	Kerato-iritis with mutton-fat deposits ..	1·4
4	Choroidal nodule .. .. .	1·25
5	Kerato-iritis with phlyctenules .. ..	1·1
6	Tubercular cyst of iris .. .. .	0·87
7	Neuro-choroidal retinitis .. .. .	0·8
8	Choroidal tubercle .. .. .	0·7
9	Interstitial keratitis .. .. .	0·7
10	Keratitis with glands in the neck .. ..	0·55
11	Keratitis with cervical and abdominal glands	0·5

was obtained in each case, though only two of them showed tubercular lesions elsewhere. In one case of recent phlyctenular keratitis the result was negative. In three cases of choroiditis in young women, free, apparently, from traces of syphilis, the reaction was positive, though no tuberculous focus could be found elsewhere. Of eight cases of interstitial keratitis, five showed obvious signs of inherited syphilis, and in these the result was negative; in the three others it was positive.

<sup>1</sup> *Gaz. Hebdomadaire de la Soc. Méd. de Bordeaux*, July 18, 1907.

<sup>2</sup> *British Medical Journal*, October 19, 1907, p. 1038

Of three cases of episcleritis, one had enlarged cervical, axillary, and inguinal glands, and the result was positive ; in the two others it was negative.

One case of tubercle of the iris, one of tubercle of the cornea, and two of chronic irido-cyclitis, also gave positive results.

Two important considerations, however, militate against its use in ophthalmic surgery. The first is that it is a test for the presence of an active tuberculous focus anywhere in the body, and, inasmuch as syphilitic keratitis and tuberculous adenitis may well be coexistent, positive result cannot be taken as indicating certain ocular tuberculosis. The second objection is that it is now regarded as inadvisable to apply the test to any but a perfectly healthy eye, and the advisability of applying it to the other eye when one is diseased is still a matter of doubt. Should this be decided upon, it is advisable to use a more dilute tuberculin than is ordinarily employed. A first application may be made of a solution of 1 in 500. If no reaction occur, a second may be made of double this strength ; beyond this it is hardly advisable to go, as several cases of severe corneal ulceration or irido-cyclitis have now been reported from the use of a 1 per cent. solution.

A positive reaction is to be considered merely as indicating an active tuberculous focus somewhere in the body. A negative reaction with a solution of 1 in 250 is not to be held as proving the absence of tuberculous infection, but only as confirmatory evidence of its absence.

As regards the variety of the tubercle bacillus at work in ocular tuberculosis we are quite ignorant. Probably it is sometimes the one variety, at another time the other.

This consideration induced me to give trial to the mixed T.R.'s in equal proportions ; the results in the few cases in which it has so far been tried have been so uniformly better than those previously obtained that I now make exclusive use of this preparation. Nor is this all ; certain cases there are, such as solitary tubercle of the choroid or iris, where toxæmia is altogether absent, and the efforts need only be directed towards the destruction of the bacilli at the infected focus ; but other cases there are, such as severe episcleritis, especially when these are secondary to tuberculosis elsewhere, in which toxæmia appears to play an important part. In these, therefore, I now combine Denys' tuberculin in conjunction with the mixed human and bovine T.R.'s, as this preparation possesses powerful antitoxin-exciting properties.

If toxæmic symptoms be absent, it is well to begin with a dose of 0·00001 c.c. of mixed human and bovine T.R.'s, and repeat in ten to fourteen days. The dose is doubled after another similar interval, and, if all goes well, repeated in about ten days. Guided by symptoms, the dosage is slowly increased, and the intervals decreased to seven to ten days.

In cases such as severe episcleritis or irido-cyclitis, combine with the T.R.'s a small quantity of Denys' tuberculin, beginning with 0·00001 c.c., and doubling this amount at each injection. The increase in dosage of this tuberculin is thus more rapid than in the case of the T.R.'s.

With females particular attention must be paid to the menstrual periods, and no inoculation should be performed within the space of time from three days prior to the onset of a period to three days after its cessation.

If treatment upon these lines be persisted in, uniformly

successful results should be secured, assuming the diagnosis to have been correct. Prolonged treatment, however, may be necessary.

Choroidal nodules may be watched disappearing by means of the ophthalmoscope; gradual shrinkage occurring until nothing is seen but a white scar, or total absorption takes place. Interstitial keratitis should clear completely, and mutton-fat deposits disappear.

### CONJUNCTIVITIS.

The forms of conjunctivitis to which vaccine therapy is especially applicable are—

1. Acute forms : Gonococcal, pneumococcal, streptococcal.

2. Chronic forms : Those due to the Morax-Axenfeld, the tubercle and Friedländer's bacillus.

In the acute forms, vaccine therapy is of especial service, in that extension to other parts can almost certainly be obviated if the case be seen early. In each of these infections a 250,000,000 dose of stock vaccine should be given as soon as the organism has been diagnosed from smears. Clinical signs are a quite sufficient guide to repetition, which may be performed in three or four days, best with a vaccine meanwhile prepared from the patient's own organisms. Such a case was one under Professor McHardy. It was seen by three members of the staff at the Royal Eye Hospital, and so bad a prognosis given that, as a last resort, I decided to give an immediate inoculation; 250,000,000 of a stock gonococcal vaccine were given, and the index found to be 2.5. Although the patient was extremely negligent of himself, and could not be induced to use a lotion or guard

to the other eye, improvement began immediately, and, despite the fact that on the fourth day the negative phase was still persisting, the index being only 1·26, marked change was evident. The active process was checked; there was much less chemosis and little discharge. Upon the eighth day the index was 3·8, and the condition of the eye so satisfactory that the patient could not be induced to make any further attendance.

Three cases of acute pneumococcal conjunctivitis, progressing to hypopyon ulcer, have also been subjected by the author to vaccine therapy. Two of these cases were under the care of Dr. Willoughby Lyle, who has very kindly furnished the following notes upon them :

‘ A male, aged forty-nine, was suffering from a rapidly infiltrating corneal ulcer, with hypopyon two-thirds up the aqueous chamber. Local treatment was persevered with for twelve days without any improvement whatever taking place; in fact, the intra-ocular tension was raised and the local pain so great (and there was no perception of light) that it was almost decided to excise the eyeball. Vaccine therapy, however, was commenced, and local treatment persevered with. In four days the hypopyon began to disappear, and the cornea to clear at the margins. From that time until the patient left the hospital—four weeks later—the eye gradually improved. Altogether two injections of 250,000,000, and one of 500,000,000, pneumococci were given. On examination two days after leaving the hospital, the local condition was as follows : There was a large irregular leukomatous patch, somewhat vascular, over the lower two-thirds of the cornea, a narrow ring of clearer cornea below the leukoma. The margin of the pupil could just be seen over the nebulous cornea above. The intra-ocular tension was

normal, and the patient could distinguish between light and darkness.

‘ The second case was in a child, aged three years seven months, who was admitted into the hospital with a central corneal ulcer with infiltrating margins and a small hypopyon. In spite of local treatment, the hypopyon increased, the ulcer spread, and the cornea ruptured. Vaccine treatment was commenced with a dose of 175,000,000 pneumococci, and from that time the eye began to clear. When the child left the hospital there was a large “leukoma adherens”; the cornea was somewhat vascular; there was a well-formed aqueous chamber; the iris was a good colour, and reacted readily to light.

‘ The favourable result obtained in these cases was very largely due to the vaccine treatment, and but for it the first-mentioned patient would undoubtedly have lost his eye.’

The third case was under the care of Mr. L. V. Cargill, and was a very severe one. As soon as the pneumococcus was isolated, a dose of 250,000,000 of the pneumococcal vaccine which had been prepared for Case 1 of Dr. Lyle’s was given. Improvement began within twenty-four hours, and progressed with extreme rapidity. The hypopyon was rapidly absorbed, and the patient discharged within a week.

Of the three forms of chronic conjunctivitis, two are, it is true, uncommon, but are singularly intractable to ordinary treatment. I refer to the forms due to the tubercle bacillus and the bacillus of Friedländer. The latter of these is especially intractable, and may persist for years; whereas improvement may be anticipated from the administration of a single dose of 250,000,000 organisms, and cure from three or four doses. The third



form, by far the commonest variety of chronic conjunctivitis, is that due to the *Bacillus lacunatus* of Morax-Axenfeld, and occasionally proves very resistant to treatment, especially in old people. Such cases should receive two or three injections of 100,000,000 organisms at intervals of seven to ten days, clinical appearances being sufficient guide as to the appropriate time for repeating the injection. Should cure not result, one or two doses of 250,000,000 organisms will almost certainly achieve this end. This organism, though easy to isolate, yields but feeble growth, and the preparation of the vaccine is no easy matter. The results are, however, so satisfactory as to afford sufficient compensation for the trouble and expense incurred.

#### CORNEAL ULCERS.

The bacteriology of these is not well known. The tubercle bacillus and pneumococcus are certain causes of some chronic varieties. Acute ulceration may be due to streptococcus, staphylococcus, gonococcus, *Bacillus coli communis*, *Bacillus pyocyaneus*, and other organisms. No matter how high the index may be to an infecting organism in these cases, immediate injection of a stock vaccine should be made as soon as the infection has been identified. The preparation of a vaccine from the patient's own bacteria should then be proceeded with, and a fresh injection be given should no response to the stock vaccine be noticeable.

Of the chronic forms due to the pneumococcus, the *ulcus serpens corneæ* is the best known. Two such cases have been treated by the author, with complete success, by means of autogenous vaccine. One of these deserves

further mention. The patient was a man, eighty years of age, under the care of Mr. Brookbanks James, and was admitted with a very bad corneal ulcer. A large hypopyon was present ; the cornea was very opaque, the iris bound down by adhesions, and the tension + 1·5. Cauterization, paracentesis, and, later, sclerotomy for the relief of tension and evacuation of the hypopyon, brought only temporary improvement, and excision seemed the only remedy. The condition was still acute when the pneumococcus was isolated and a vaccine prepared. Despite the high index to this organism—viz., 2·5—an injection of 250,000,000 organisms was given. Within three days the eye began to improve in appearance ; at the end of a week the index was 4·2, and after a fortnight 3·0. A second injection was then given, with the result that, eighteen days later, the index stood at 6·3, and the inflammation had quite subsided. A large partial staphyloma of the cornea which developed later was treated radically by excision without the use of sutures. No reaction followed the operation, and the final result was an eye in which some slight vision was preserved, and a firm flat scar in the cornea left in the site of the staphyloma. Several months later the eye was quite quiet and free from irritability.

The following case of ulcerative keratitis, under the care of Professor McHardy, at the Royal Eye Hospital, is not without interest : The patient had already had one eye removed for chronic ulcerative keratitis, going on to perforation, shrinking of the globe, and considerable pain. About eighteen months subsequently the second eye was also attacked ; general superficial erosion of the cornea went on to infiltration of the more superficial, then of the deeper layers of the cornea ; the tension fell considerably

and vision was practically nil, only dim perception of light being possible. Cultures from the surface of the globe yielded large cocci not staining by Gram's method, which were apparently not the *Micrococcus catarrhalis*, and diplococci which morphologically resembled the pneumococcus, but could not be isolated. Upon the chance of the infection being a pneumococcal one, two injections of a vaccine were given without producing any good result; on the contrary, the condition became rather worse. The vision was so bad that an iridectomy was decided on. The iris, when seized by the forceps, simply tore at once, it was so pulpy; cultures were made from this small portion of iris and from scrapings of an eroded portion of the cornea, and the same non-Gram-staining coccus obtained from both. The organism so far has not been identified, but a vaccine was made of it as a last hope. A first injection of 250,000,000 organisms, the index being 0·5, was followed, ten days later, when the index was 0·8, by a second of like amount; seventeen days after the first injection, the index being 1·5, the eye began definitely to improve, and steadily continued to do so. Twenty-eight days after the second injection the index was 2·6, and a further dose of 400,000,000 given. The eye was now very much better; vision was returning, fingers being seen at about 1 foot. In another twenty-six days the patient could discern faces fairly well, and a further injection of 500,000,000 cocci was given. A fortnight later vision was further improved, and the patient was discharged. When seen two months later, vision, both near and for distance, =  $\frac{1}{60}$ ; the cornea was diffusely nebulous; the eye was quite quiet, and vision had decidedly improved since the patient had left the hospital.

Mackay described before the Ophthalmological Society

a case of phlyctenular keratitis. Tubercle bacilli could not be found, and the tuberculo-opsonic index was within normal limits. The staphylococcal index being 1·24, treatment with this vaccine was begun; improvement began immediately, but recurrences occurred when too long intervals were left between the inoculations.

The patient has remained well since completion of the treatment.

Brief reference may finally be made to the assistance afforded the surgeon in such conditions as recurrent 'hordeolum' and meibomians, for which staphylococci are responsible, and in chronic dacryocystitis, which, as Eyre has shown, is practically always due to the *Streptococcus pyogenes longus*, whether subsequent to acute streptococcal conjunctivitis or to one of the other acute forms; in this instance doses of 100,000,000 organisms may be begun with, and repeated or increased at intervals, best controlled by determinations of the index, or, in default of these, by the clinical appearances.

## CHAPTER XIII

INFECTIONS DUE TO THE MICROCOCCUS MELITENSIS, BACILLUS PARALYTICANS, MICROCOCCUS NEOFORMANS, MENINGOCOCCUS, STREPTOTHRIX ACTINOMYCOSIS, BACILLUS PYOCYANEUS, LEPROBACILLUS, BORDET'S BACILLUS OF WHOOPING-COUGH. PYORRHOEA ALVEOLARIS.

### THE MICROCOCCUS MELITENSIS.

IN comparatively light attacks, when the fever is likely to run on for months without any severe intoxication of the system, and where the imperfect development of the agglutination reaction seems to indicate that the immunizing impulses are in default, Wright advocates the use of a vaccine.

He has successfully treated a case of localized infection supervening upon an attack of Malta fever.

Bassett Smith,<sup>1</sup> following up the apparently successful treatment by Reid of nine cases of Malta fever by means of a vaccine, observed the results of such treatment in sixty - one cases, to which 224 injections were given. These cases comprised all grades in the disease, from the severe undulant type to the intermittent. The initial dose employed was usually about 50,000,000 organisms, and this was but rarely exceeded, the interval between the injections being ten days. The negative phase was frequently very short or altogether absent, a steady rise being commonly observed.

<sup>1</sup> *Journal of Hygiene*, January, 1907, p. 115.

No relationship was found to exist between the curves of the opsonic indices and the agglutination reactions of the patient's sera. Bassett Smith concluded that the vaccine treatment of Malta fever appears in a certain number of cases to produce a beneficial result, the severity of the symptoms being diminished, the general condition improved, and the duration of the disease curtailed ; but that in the more severe type of case, with high fever and evidence of severe intoxication, the method appears to have a deleterious instead of a favourable action.

I would suggest that the more frequent administration of much smaller doses, as in streptococcal and gonococcal septicæmias, might possibly secure more favourable results.

#### THE BACILLUS PARALYTICANS.

Numerous attempts have been made to isolate from the blood and cerebro-spinal fluid of cases of general paralysis of the insane and of tabes dorsalis an organism or organisms which might prove to stand in a causal relationship to this disease or to the congestive seizures.

Ford Robertson and McRae claimed to have demonstrated the constant presence of an organism of the diphtheroid group, to which they gave the name *Bacillus paralyticans*, in the blood, cerebro-spinal fluid, and brain tissues. Other observers, among whom may be mentioned Eyre and Flashman, have, however, shown that there is hardly any part of the body where diphtheroid organisms had been obtained by Robertson in cases of general paralysis from which similar organisms could not be obtained in cases free from any semblance of insanity.

The difficulties in technique are so great, and the risks of contamination in taking cultivations so considerable, that variable results are almost inevitable in the hands of different observers. It may be mentioned that the psychological moment for taking cultures is as soon as possible after a congestive attack, for leucocytosis is rapidly developed, and in an hour or two the phagocytic action of the leucocytes may destroy all the organisms in the blood-stream. Sufficient attention to this point does not appear to have been paid by all observers. Ford Robertson now considers that a second organism, to which he has given the name *Bacillus paralyticans brevis*, is also concerned in the production of a certain proportion of cases of general paralysis. Candler<sup>1</sup> altogether failed on forty-one occasions in twenty-four cases to find either of these organisms, while G. M. Robertson<sup>2</sup> upon fifteen occasions in seven cases of undoubted general paralysis recovered a diphtheroid organism from the blood or cerebro-spinal fluid, which, however, appeared to differ from either of Ford Robertson's forms. Sufficient evidence has not yet been accumulated to warrant the view that any definite member of the diphtheroid group is responsible for the production of general paralysis, although it seems likely that the presence of these organisms is more than a coincidence, and important discoveries may soon be anticipated.

O'Brien<sup>3</sup> details the result of opsonic determinations with Ford Robertson's original *Bacillus paralyticans* upon seven cases of this disease. The indices showed great fluctuations, leading him to the conclusion that the infection is a systematic one. Injections of a vaccine

<sup>1</sup> *Lancet*, August 17, 1907, p. 450.

<sup>2</sup> *Ibid.*, p. 449.

<sup>3</sup> *Journal of the American Medical Association*, 1906, p. 2180.



were given about every fourteen days, and marked improvement in the symptoms claimed to be noticed.

These results must be received with great caution, in view of Ford Robertson's modified opinion of the causal relationship of this organism to the disease, and in consideration of the fact that remissions in the course of general paralysis are very common. In so dread a disease no chance of doing good should, however, be neglected, and, in the event of the isolation of a diphtheroid organism from cultures of the cerebro-spinal fluid taken during or immediately after a congestive attack, the administration of a vaccine prepared from this organism would appear to be a justifiable—nay, advisable—proceeding.

#### THE MICROCOCCUS NEOFORMANS.

The contention of Doyen that this organism is the true cause of carcinomatous tumours has not been accepted by pathologists in this country; for not only is it found in the vicinity of carcinomata, but also in that of sarcomata and such benign growths as adenomata. Inoculation experiments upon rats and mice have also completely failed to produce a malignant tumour.

A case of cancer of the larynx was treated by Wright with this vaccine. Although it appeared to improve for a time, death ensued in about six months, and was found post-mortem to be due to cancer.

Jacobs and Geets<sup>1</sup> recorded the results of treatment in thirty-seven cases of mammary carcinoma. They regard the *Micrococcus neoformans* as the cause of the cancerous cachexia. The index in these cases was found usually to be below 0·8. Cases in which the index failed to rise

<sup>1</sup> *Lancet*, April 7, 1906, p. 964.

after two injections they considered hopeless from exhaustion of the defensive powers.

The results of treatment seem to be decrease of surrounding infiltration, reduction in the size of the nodules, which usually become freely movable, great improvement in the patient's appearance and general condition, and diminution of pain. This they hold to be the time for operation. They tabulate their results as follows :

' Cure ' maintained after several months in					7 cases.
Lasting improvement in	..	..	..	12	„
Transient result in	..	..	..	7	„
No result in	..	..	..	11	„
Total	..	..	..	37	„

The opinion at the London Cancer Hospital is that as a curative agent a vaccine of the *Micrococcus neoformans* is valueless, and its employment has therefore been entirely abandoned at that institution. It may, however, well be left to any inoperable case of cancer to decide whether employment of this harmless agent shall be made, in the remote hope of the case being brought within the zone of operability. Admitting that the *Micrococcus neoformans* is not the cause of cancer, it cannot, however, be denied that it is almost always associated with the cancerous tumour, and that injections of 25,000,000 to 50,000,000 occasionally benefit the local and general condition.

#### THE MENINGOCOCCUS.

Hector Mackenzie<sup>1</sup> has treated a case successfully by means of an autogenous vaccine. A first dose of 120,000,000 was followed in ten days by one of 20,000,000, in a week later by one of 143,000,000, and in another week by a final dose of 71,000,000.

<sup>1</sup> *British Medical Journal*, 1907, vol. i., p. 1408.

Rundle and Mottram<sup>1</sup> have recorded a successful result by vaccine treatment in a case in which the prognosis was distinctly bad. The index was 0·7 when an injection of 200,000 organisms was given; next day it had risen to 1·5. In the subsequent twenty days four doses of 500,000 organisms were given, each followed by negative and positive phases. Recorded cases of successful treatment are so far very few, but I have heard privately of two other such instances; while Bosanquet and Eyre have had three failures. The success attending the intraspinal use of Flexner and Jobling's serum, and of that prepared by Kolle and Wassermann, appears to be so considerable as to indicate that this is the best method of combating infection by this organism. About 20 c.c. of cerebro-spinal fluid are withdrawn by spinal puncture, and replaced with a like, or slightly less, volume of the serum.

Houston and Rankin,<sup>2</sup> Fordyce,<sup>3</sup> and others, have studied the opsonic and agglutinative power of the blood-serum and cerebro-spinal fluid in cases from various epidemics. With normal serum phagocytosis is extremely slight, and does not occur until degeneration of the coccus has commenced. This is true, irrespective of whether the organism has been freshly isolated or repeatedly subcultured. With 'immune' sera the degree of phagocytosis very rapidly increases with the extent to which the organism has been subcultured. The difficulty of some observers with clumping of the emulsion is very greatly obviated by employing a growth six to ten hours old and incubating the opsonic mixture for

<sup>1</sup> *Lancet*, July 27, 1907, p. 220.

<sup>2</sup> *British Medical Journal*, November 16, 1907.

<sup>3</sup> 'International Clinics' (eighteenth series), vol. i., p. 40.

only ten minutes. Employing this technique, the author has not experienced the slightest trouble, even with the most freshly isolated organisms.

At the very onset the index is probably low. Thus, in one instance Fordyce found an index of only 0·4 within twenty-four hours of onset. Later the index of the blood-serum rises very rapidly, and within five or six days may be between 5 and 10. Later it may be as high as 30 or 40. The agglutinative power of the serum is also very marked. In convalescent cases these typical reactions disappear rapidly. The cerebro-spinal fluid has very much lower opsonic and agglutinative power than the blood-serum, and may even have none at all; Houston and Rankin conclude that these reactions are of very great value in the diagnosis of early cases of true epidemic cerebro-spinal meningitis, and in differentiating them from those of posterior basic meningitis, which are due to a Gram-negative coccus very closely resembling the true meningococcus, both morphologically and culturally.

Owing to the rapid loss of virulence on the part of this organism, if a vaccine be employed it should be prepared from a first subculture, if possible, and preferably from the patient's own organism.

The appropriate dosage is as yet uncertain. It is perhaps advisable to begin with 5 to 10 millions, and repeat and increase as the index or clinical symptoms indicate.

#### ACTINOMYCOSIS.

The first case of actinomycotic, or rather streptotrichotic, disease treated by means of a vaccine has been that of the lung, and probably of the liver, described

by Wynn.<sup>1</sup> The infection probably dated back at least twelve months, and six months prior to admission to hospital extension seems to have occurred from the bronchi to the lung tissue, and much sputum with a feculent odour was expectorated. Subsequent formation of an empyema required operation, and from the pus a pure culture of streptothrix was isolated, and a vaccine prepared from a forty-eight-hours-old agar culture. The dose employed for each inoculation represented 0·001 milligramme of bacterial substance. Attempts were made to estimate the index, which was approximately 0·3 on January 3 and 0·5 on January 7; on January 8 the first inoculation of 0·001 milligramme was given. Twenty-four hours later the negative phase was apparently over, as the index had risen to 0·7, and by January 16 was 1·2. In a few days the cough became less troublesome, and the sputum and discharge of pus diminished in a remarkable way. The temperature dropped from over 100° F. to normal, and remained normal for three days. Four days after injection the discharge had so diminished that the drainage-tube was removed. A slight rise of temperature resulted, and on the 18th instant a second inoculation of 0·001 milligramme was given. Three days later temperature was again normal, and remained so. Subsequent injections were given on February 11 and 25, and March 11 and 27, each of 0·001 milligramme. The patient gained 1 stone 6 pounds in weight, and the condition on discharge was a thickened pleura, with a large, dry cavity in the lung. There was no sputum, and only occasionally a dry cough. The patient has continued well.

Short<sup>2</sup> points out that streptothricial infection of the

<sup>1</sup> *British Medical Journal*, March 7, 1908.

<sup>2</sup> *Lancet*, September 14, 1907, p. 760.

human subject is much commoner than usually supposed, and that probably over 2 per cent. of all cases of perityphlitis are due to it. The disease, though very chronic, ends fatally in quite 60 per cent. of the cases within nine months. He also describes a case of actinomycosis of the lungs in which the signs were those of broncho-pneumonia, except that the dulness was uniform. The temperature was high, and the result fatal within a few weeks.

In view of Wynn's success with vaccine therapy, the importance of making careful search for granules and mycelia in obscure lung and appendicular cases is obvious.

#### THE BACILLUS PYOCYANEUS.

The successful treatment of a case of pyæmia due to this organism is recorded by Groves.<sup>1</sup> There was a history of hip-joint trouble dating back nine months. An operation was performed, and the head of the femur and the socket found to be eroded. The day after the temperature rose to 103° F., and remained there for five days, when it began to fluctuate 3° or 4°, rising as high as 104° F. Delirium, emaciation, and multiple abscesses, followed. Six weeks after operation the *Bacillus pyocyaneus* was recovered in pure culture from the sanious discharge. A vaccine was made, and 40,000,000 given, without any appreciable result. Eight days later, when the temperature was 101·5° F., 60,000,000 were given. Within two days the temperature fell to normal, and remained there. At intervals of ten, fifteen, twenty-three, and fifteen days, respectively, injections of

<sup>1</sup> *British Medical Journal*, May 15, 1909, p. 1169.



100,000,000 were given. From the date of the second injection the whole condition rapidly improved: quiet sleep was secured, and food was taken well; weight was put on rapidly, and all the abscesses and sinuses healed, except one quite small superficial one. The surgeon concluded that a more striking example of the potency of vaccine therapy could hardly be imagined.

#### LEPRA BACILLUS.

For a full description of an attempt by Dr. Nichols to treat a case of leprosy in a patient of Sir Malcolm Morris by means of a vaccine, see Proceedings of the Royal Society of Medicine, Dermatological Section, p. 41, December, 1908. A nodule was excised and placed in a tube of glycerine broth. After a fortnight's incubation it was slowly desiccated and ground up. This vaccine was standardized in the usual way, and doses of 50,000,000 to 300,000,000 employed. There was distinct temperature reaction after each dose, and ultimately considerable apparent improvement in the nodular condition.

#### BORDET'S BACILLUS OF WHOOPING-COUGH.

The results of the vaccine treatment of whooping-cough would appear to indicate that the micro-organism described by Bordet and Jochmann stands in a causal relationship to the disease. Martha Wollstein<sup>1</sup> has re-studied this organism. In early cases it appears in great numbers in the secretions, often associated with the *Bacillus influenzae*, and is especially easily recovered from the lungs at autopsy. After the second week it is not

<sup>1</sup> *Journal of Experimental Medicine*, January, 1909, p. 41.



present in sufficient numbers to be easily isolated, whereas the *B. influenzae* may persist in considerable numbers. It is a short, poled, ovoid bacillus, slightly larger than the *B. influenzae*, non-motile, very regular in size and shape, arranged singly or in pairs, either end to end or side by side. It is negative to Gram's stain, and colours especially well with toluedene blue. While the poles stain deeply, a pale area remains in the centre, and this is a very constant and well-marked feature, especially in young cultures. The best medium for its cultivation is Bordet's glycerine-potato blood-agar, plates of which are inseminated thinly with flakes of secretion which have been well washed in sterile water. After twenty-four hours' incubation, colonies of *B. influenzae* may be seen; upon the second or third day much larger, vigorous colonies appear, which are the Bordet bacillus. These, unlike the *B. influenzae*, do not hæmolyze the medium. The colonies after forty-eight hours' incubation are small, discrete, and greyish; later they increase in thickness rather than in size. After the first generation upon glycerine-potato blood-agar they are dense, grey, glistening, and much more vigorous than the *B. influenzae*. Upon ordinary blood-agar they much resemble the latter. They are strictly aerobic. Bordet and also Freeman<sup>1</sup> have found that the serum of patients suffering from the disease agglutinates the organism, sometimes in as much as a sixty-four-fold dilution. The complement-deviation method also confirms the relationship.

Bordet inoculated children with a vaccine, and produced a marked reaction. Freeman has performed over 1,000 inoculations in children with whooping-cough, and found that the result with a dosage of 25,000,000 appar-

<sup>1</sup> *Lancet*, August 14, 1909, p. 471.

ently was to reduce the duration of the disease by nearly one-half. My own experience would seem to indicate that doses of 50,000,000 to 100,000,000 every four or five days produce an even better effect.

### PYORRHŒA ALVEOLARIS.

To few infective processes has more bacteriological study been devoted than to this exceedingly common and revolting complaint; the elucidation thereby thrown upon its ætiology is correspondingly slight. The only conclusion I can arrive at after three years' close study is that the causative organisms are many, varying in nearly every instance. That the ordinary mouth organisms are closely connected with the process is beyond doubt. Personally I think that in very early cases the staphylococci, *Streptococcus longus* and *brevis*, *Micrococcus catarrhalis*, and pneumococcus, among others, play an important part, but that in advanced stages these are commonly supplanted by the spirochætes and spirillæ, broth cultures of which are intensely toxic, and contain an active hæmolysin, to which the anæmia often associated with the disease is to be attributed.

In twenty-four cases Eyre found the *Streptococcus pyogenes* seven times, the *Micrococcus catarrhalis* eight times, a mixture of these two six times, and the pneumococcus three times. He recommends 5,000,000 to 10,000,000 of the appropriate vaccine to be injected every six to eight days, treatment being prolonged for three to six months. Goadly<sup>1</sup> reports two cases successfully treated with a *Staphylococcus aureus* vaccine.

Vaccine treatment, in my opinion, is only to be regarded

<sup>1</sup> *British Medical Journal*, 1908, vol. ii., p. 477.

as a very useful adjuvant to local treatment carried out carefully and methodically, as it is by a very few of the dental profession. The combined is a more speedy and effectual method of treatment than by vaccines alone. Its result is also more lasting, for if vaccine treatment succeed in sterilizing a deep pocket, it fails to cause a re-approximation of the gum to the tooth, and reinfection is inevitable sooner or later. Nevertheless, in refractory cases the assistance of a vaccine is not to be despised, as the good effect thereby produced upon the constitutional symptoms is frequently very marked.

## CHAPTER XIV

### ANTIPLAGUE AND ANTICHOLERA VACCINATION

THE vaccine therapy of infections by the *Bacillus pestis* and *Vibrio cholerae* is at present confined to preventive inoculation as devised and perfected by Haffkine. The *Bacillus pestis* produces a powerful endotoxin, a true exotoxin apparently being also produced, but in small amounts. Immunity appears to be due to the production of bacteriolysin. According to Wright, the human blood possesses no bactericidal action, recovery being due to opsonic action and phagocytosis. Haffkine's plague prophylactic is prepared by growing the bacillus in flasks of broth to which a few drops of oil are added ; to these latter the bacteria attach themselves, and form colonies, which hang down into the broth in the form of stalactites. By shaking the flasks from time to time these are detached, and others form in their places. After four to six weeks' incubation at 27° to 30° C., the cultures are sterilized by heating at 65° C. for one hour ; their sterility is tested by cultures, and 0·5 per cent. carbolic acid then added. The dose of this vaccine is 3 c.c. for an adult man, 2·0 to 2·5 c.c. for a woman, and smaller doses for children, according to their age. Local and constitutional symptoms of moderate severity ensue, and last for twenty-four to forty-eight hours, but do not as a rule necessitate the abandonment of ordinary

occupation. Immunity appears to be produced with rapidity, perhaps even within twenty-four hours, and lasts for twelve to eighteen months. Not only is the liability to infection much diminished, but also the case mortality. Thus, Haffkine<sup>1</sup> states that among natives the liability to infection is diminished to less than one-third of that occurring among the uninoculated, and that the case mortality is reduced by more than 50 per cent.; so that the total reduction of the death-rate among the inoculated is about 85 per cent. He also asserts—(1) that if in a European an attack subsequently occur, it always ends in recovery; (2) that inoculation is applicable to persons already infected and incubating the plague, and that thereby symptoms are altogether prevented or much mitigated; (3) that in natives the degree of immunity conferred appears to last through several outbreaks, whilst in Europeans it does not seem to have yet disappeared during the ten years since the inauguration of the method.

The general opinion in India is that absolute safety is insured for at least three months. As with antityphoid inoculation, a second administration of the vaccine in ten to fourteen days appears to confer an additional immunity.

Pfeiffer advises that the vaccine be prepared from two-day-old agar cultures, emulsified with broth or salt solution, and sterilized at 65° C. for one hour; while Strong has suggested the use of living attenuated bacilli, and employed such a vaccine at Manila without any ill results. Whether either of these vaccines yields better results than those obtained by the use of Haffkine's prophylactic has not yet been determined.

<sup>1</sup> Proceedings of the Royal Society of Medicine, December 2, 1907.

In view of the likelihood that the first result of an inoculation with any of these vaccines is to produce an increased susceptibility, the simultaneous administration of 10 to 25 c.c. of Yersin's or of Lustig's antitoxic serum has been recommended.

As in the case of the *Bacillus pestis*, the *Vibrio cholerae* forms a very powerful endotoxin, and but little exotoxin; but whereas plague is a septicæmic disease, cholera is a strictly localized infection of the intestinal contents, living bacteria apparently gaining no foothold in the tissues, unless it be that bacteriolysis of the vibrios in the blood occurs with such rapidity as to prevent their recognition therein. So far as is definitely known at present, immunity depends entirely upon bacteriolysis, no antitoxic substances being present either in the serum of convalescent patients or of immunized animals.

In anticholera vaccination as practised by Haffkine, use is made of two vaccines—a weak one and a strong one. The weak vaccine is prepared by growing an ordinary laboratory culture in broth at 39° C. in a current of air; its sterility is tested day by day until subcultures upon agar no longer show growth. The last subculture that grew is then taken and grown on agar slopes 10 centimetres long at 39° C., in a current of air. After twenty-four hours' incubation, 8 c.c. of broth or 6 c.c. of 0·5 per cent. carbolic acid solution are added to each tube, and a thorough emulsion prepared. The dose of this weak vaccine is 1 c.c. The strong vaccine is prepared in a similar way from the vibrios after passage through a series of guinea-pigs, this process being continued from animal to animal until a culture is obtained which is invariably fatal within eight hours. This final 'virus fixe' is used to inseminate agar slopes as before, the

incubation being, however, carried on at 35° C. instead of at 39° C., as the former is the optimum temperature for the *Vibrio cholerae*. The dose of this strong vaccine is also 1 c.c. One or two injections of the weaker vaccine are made subcutaneously into the flank at intervals of three to five days, a dose of the stronger being given five days after the last of the weaker. The injections cause moderate pyrexia, general malaise, and headache, while locally tenderness and swelling of the corresponding lymph glands result. These symptoms all pass off in about three days.

Kolle<sup>1</sup> advises the use only of a vaccine of the fully virulent organisms, and finds that the local necrotic effects which were anticipated do not occur. He claims that more rapid immunity results. The full immunity from each inoculation is attained in about five days, so that it is complete in about ten days, and is said to last for at least a year. The good results are seen in a reduction of the incidence of the disease, and not of the case mortality, which appears to be uninfluenced. This may be seen in the table given below, which is compounded of the recorded results from numerous sources spread over several years :

TABLE XXII.

	Population.	Cases.	Deaths.	Fatality.
Uninoculated..	18,355	667 = 3·6%	393 = 2·1%	59%
Inoculated ..	12,744	84 = 0·66%	48 = 0·4%	57%

<sup>1</sup> *Centralb. für Bact.*, 1896, Bd. xix.



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